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=> s association constant
L1 28669 ASSOCIATION CONSTANT

=> s l1 and therapeutic potential
L2 5 L1 AND THERAPEUTIC POTENTIAL

=> dup remove l2
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L3 2 DUP REMOVE L2 (3 DUPLICATES REMOVED)

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L3 ANSWER 1 OF 2 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
1999:179657 The Genuine Article (R) Number: 172BQ. Poly(L-lysine)-graft-dextran copolymer promotes pyrimidine motif triplex DNA formation at physiological pH - Thermodynamic and kinetic studies. Torigoe H; Ferdous A; Watanabe H; Akaike T; Maruyama A (Reprint). Inst Phys & Chem Res, RIKEN, Tsukuba Life Sci Ctr, Gene Bank, 3-1-1 Koyadai, Tsukuba, Ibaraki 3050074, Japan (Reprint); Inst Phys & Chem Res, RIKEN, Tsukuba Life Sci Ctr, Gene Bank, Tsukuba, Ibaraki 3050074, Japan; Tokyo Inst Technol, Dept Biomol Engn, Yokohama, Kanagawa 2268501, Japan. JOURNAL OF BIOLOGICAL CHEMISTRY (5 MAR 1999) Vol. 274, No. 10, pp. 6161-6167. ISSN: 0021-9258. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Extreme instability of pyrimidine motif tripler DNA at physiological pH severely limits its use for artificial control of gene expression in vivo. Stabilization of the pyrimidine motif tripler at physiological pH is therefore of great importance in improving its **therapeutic potential.** To this end, isothermal titration calorimetry interaction analysis system and electrophoretic mobility shift assay have been used to explore the thermodynamic and kinetic effects of our previously reported tripler stabilizer, poly (L-lysine)-graft-dextran (PLL-g-Dex) copolymer, on pyrimidine motif tripler formation at

physiological pH. Both the thermodynamic and kinetic analyses have clearly indicated that in the presence of the PLL-g-Dex copolymer, the binding constant of the pyrimidine motif tripler formation at physiological pH was about 100 times higher than that observed without any tripler stabilizer. Of importance, the tripler-promoting efficiency of the copolymer was more than 20 times higher than that of physiological concentrations of spermine, a putative intracellular tripler stabilizer. Kinetic data have also demonstrated that the observed copolymer-mediated promotion of the tripler formation at physiological pH resulted from the considerable increase in the association rate constant rather than the decrease in the dissociation rate constant. Our results certainly support the idea that the PLL g-Dex copolymer could be a key material and may eventually lead to progress in therapeutic applications of the antigene strategy in vivo.

L3 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 1
85081370. PubMed ID: 2578164. Non-cross-reactive monoclonal antibodies to human chorionic gonadotropin generated after immunization with a synthetic peptide. Caraux J; Chichehian B; Gestin C; Longhi B; Lee A C; Powell J E; Stevens V C; Pourquier A. Journal of immunology (Baltimore, Md. : 1950), (1985 Feb) 134 (2) 835-40. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Noncross-reactive monoclonal antibodies specific for human chorionic gonadotropin (hCG) were obtained after pre-selection for submolecular specificity with a synthetic peptide immunogen. Mice were immunized with a synthetic peptide representing a segment unique to the beta-subunit of hCG (amino acid residues 109-145), conjugated to diphtheria toxoid. We then derived nine different hybridomas that secreted monoclonal antibodies reactive with both native hCG and isolated C-terminal peptide, after somatic cell hybridization of immune spleen cells with a nonsecretory myeloma cell line. None of the nine monoclonal antibodies, termed beta-hCG-CTPa1----a9, reacted with hLH, hFSH, or hTSH, although these pituitary hormones display extensive amino acid sequence homology with hCG. The noncross-reactive anti-beta-hCG monoclonal antibodies show apparent **association constants** on the order of 10(9) to 10(10) M-1. A sandwich-type enzyme-linked immunosorbent assay was set up with cut-off values of around 5 mIU/ml. These antibodies might have important implications for: a) improving the diagnosis and clinical management of pregnancy; b) monitoring the course of development of carcinomas which secrete the hormone, through in vitro assays or in vivo radioimmunodetection; c) evaluating the antibodies' **therapeutic potential** against such carcinomas; d) studying the biologic functions of the C-terminal segment of beta-hCG; and e) addressing the anti-fertility effect of antibodies raised against that segment.

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L5            1 L4 AND IMPROVE

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L5   ANSWER 1 OF 1     MEDLINE on STN
87162857.     PubMed ID: 3030851.   Dynamics of LHRH binding to human term placental cells from normal and anencephalic gestations. Belisle S; Lehoux J G; Bellabarba D; Gallo-Payet N; Guevin J F. Molecular and cellular endocrinology, (1987 Feb) 49 (2-3) 195-202. Journal code: 7500844. ISSN: 0303-7207. Pub. country: Netherlands. Language: English.
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AB In order to **improve** our knowledge on human placental hCG production, we studied the binding of an LHRH agonist (N-Ac-Pro1,D-Leu6)-LHRH to third trimester intact placental cells from normal and anencephalic fetuses. In normal pregnancies, specific and saturable binding was found for both LHRH and its analogs with two classes of binding sites. **Association constants** were 4.7 +/- 2.2 (mean +/- SEM) X 10(5) M-1 for the low affinity sites and 1.7 +/- 0. **8 X 10**(8) M-1 for the higher affinity sites (P less than 0.01), and the estimated number of sites was 1.71 +/- 0.52 nmol/mg of cell protein and 2.79 +/- 0.54 pmol/mg of cell protein, respectively. Preincubation with increasing concentrations of LHRH agonist induced a progressive decrease in specific binding sites and manifested by a reduction in hCG production which paralleled the concentration of the agonist in preincubation buffer. Studies with placental cells from three anencephalic fetuses showed a decreased binding capacity for LHRH and its agonist, when compared to normal trophoblastic cells, as well as a reduced capacity to produce hCG. Our results suggest that mechanisms dependent upon LHRH binding to its receptor are required for placental hCG production in normal pregnancies. Furthermore our investigation suggests a role for the endocrine feto-placental milieu in the manifestation of these placental LHRH binding sites.

=> s l1 and modified peptide
L6 2 L1 AND MODIFIED PEPTIDE

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L7 2 DUP REMOVE L6 (0 DUPLICATES REMOVED)

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L7 ANSWER 1 OF 2 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
2000:682451 The Genuine Article (R) Number: 352DN. Inhibition of HIV-1 protease by a boron-modified polypeptide. Pivazyan A D (Reprint); Matteson D S; Fabry-Asztalos L; Singh R P; Lin P; Blair W; Guo K; Robinson B; Prusoff W H. Yale Univ, Sch Med, Dept Pharmacol, New Haven, CT 06510 USA (Reprint); Washington State Univ, Dept Chem, Pullman, WA 99164 USA; Bristol Myers Squibb Co, Pharmaceut Res Inst, Dept Virol, Wallingford, CT 06492 USA. BIOCHEMICAL PHARMACOLOGY (1 OCT 2000) Vol. 60, No. 7, pp. 927-936. ISSN: 0006-2952. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Six boronated tetrapeptides with the carboxy moiety of phenylalanine replaced by dihydroxy-boron were synthesized, and their activities against human immunodeficiency virus 1 (HIV-1) protease subsequently investigated. The sequences of these peptides were derived from HIV-1 protease substrates, which included the C-terminal part of the scissile bond (Phe-Pro) within the gag-pol polyprotein. Enzymatic studies showed that these compounds were competitive inhibitors of HIV-1 protease with K-i values ranging from 5 to 18 mu M when experiments were performed at high enzyme concentrations (above 5 x 10(-8) M); however, at low protease concentrations inhibition was due in part to an increase of the **association constants** of the protease subunits. Ac-Thr-Leu-Asn-PheB inhibited HIV-1 protease with a K-i of 5 mu M, whereas the non-boronated parental compound was inactive at concentrations up to 400 mu M, which indicates the significance of boronation in enzyme inhibition. The boronated tetrapeptides were inhibitory to an HIV-1 protease variant that is resistant to several HIV-1 protease inhibitors. Finally, fluorescence analysis showed that the interactions between the boronated peptide Ac-Thr-Leu-Asn-PheB and HIV-1 protease resulted in a rapid decrease of fluorescence emission at 360 nm, which suggests the formation of a compound/enzyme complex. Boronated peptides may provide

useful. reagents for studying protease biochemistry and yield valuable information toward the development of protease dimerization inhibitors. BIOCHEM PHARMACOL 60;7:927-936, 2000. (C) 2000 Elsevier Science Inc.

L7 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
1960:129714 Document No. 54:129714 Original Reference No. 54:24952g-i Modification of the methionine residue in the peptide component of ribonuclease-S. Vithayathil, Paul J.; Richards, Frederic M. (Yale Univ.). Journal of Biological Chemistry, 235, 2343-51 (Unavailable) 1960. CODEN: JBCHA3. ISSN: 0021-9258.
AB cf. CA 54, 18634i. At pH 2 the reaction of iodoacetic acid or iodoacetamide with the peptide component of subtilisin-modified ribonudease is limited to the methionyl residue in position 17, and results in the formation of the corresponding sulfonium salts. These peptide derivs. show a markedly reduced ability to bind to the protein component. Such **modified-peptide**:protein complexes, however, once formed, show a kinetic behavior very similar in all respects to the complex of the unmodified components. Oxidation of the methionyl residue to the sulfone has less effect on the binding constant From kinetic measurements it is inferred that cytidine 2',3'-phosphate is bound by the protein component only slightly less than by the catalytically active protein-peptide complexes. The possibility that the methionyl residue contributes to the **association consts.** through hydrophobic bonding is discussed. No evidence was found that substitution of a carboxymethyl group on the histidyl residue in position 16 has any effect on the potential activity of the peptide.

=> s method optimizing therapeutic potency
L8 0 METHOD OPTIMIZING THERAPEUTIC POTENCY

=> s optimizing therapeutic potency
L9 0 OPTIMIZING THERAPEUTIC POTENCY

=> s therapeutic potency
L10 795 THERAPEUTIC POTENCY

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L11 81 L10 AND IMPROVE

=> s l11 and amino acid substitution
L12 0 L11 AND AMINO ACID SUBSTITUTION

=> s l10 and association rate
L13 1 L10 AND ASSOCIATION RATE

=> d l13 cbib abs

L13 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2006 ACS on STN
2002:353476 Document No. 136:350534 Methods for producing and improving **therapeutic potency** of binding polypeptides. Huse, William D. (Applied Molecular Evolution, Inc., USA). PCT Int. Appl. WO 2002036615 A2 20020510, 82 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US46248 20011030. PRIORITY: US 2000-2000/702140 20001030.
AB The invention provides a binding polypeptide, or functional fragment thereof, comprising a kon of at least about 9 x 107 M-1 s-1 for associating with a ligand and having **therapeutic potency.** The invention also provides a method of determining the **therapeutic**

potency of a binding polypeptide. The methods consist of (a) contacting a binding polypeptide with a ligand; (b) measuring **association rate** for binding between the binding polypeptide and the ligand, and (c) comparing the **association rate** for the binding polypeptide to an **association rate** for a therapeutic control, the relative **association rate** for the binding polypeptide compared to the **association rate** for the therapeutic control indicating that the binding polypeptide will exhibit a difference in **therapeutic potency** correlative with the difference between the **association rates.**

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=> s association rate
L14       9828 ASSOCIATION RATE

=> s l14 and improvement
L15         56 L14 AND IMPROVEMENT

=> s l15 and selection
L16          6 L15 AND SELECTION

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L17          2 DUP REMOVE L16 (4 DUPLICATES REMOVED)

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L17  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2006 ACS on STN
2005:1089103  Ultra-potent antibodies against respiratory syncytial virus:.
     Wu, H.; Pfarr, D. S.; Tang, Y.; An, L-L.; Pate, N. K.; Watkins, J. D.;
     Huse, W. D.; Kiener, P. A.; Young, J. F. (USA). Assay and Drug
     Development Technologies, 3(4), 450-452 (English) 2005. CODEN: ADDTAR.
     ISSN: 1540-658X. Publisher: Mary Ann Liebert, Inc..
AB   We describe there the selection of ultra-potent anti-respiratory
     syncytial virus (RSV) antibodies for preventing RSV infection. A large
     number of antibody variants derived from Synagis (palivizumab), an anti-RSV
     monoclonal antibody that targets RSV F protein, were generated by a
     directed evolution approach that allowed convenient manipulation of the
     binding kinetics. Palivizumab variants with about 100-fold slower
     dissociation rates or with fivefold faster association rates
     were identified and tested for their ability to neutralize virus in a
     microneutralization assay. Our data reveal a major differential effect of
     the association and dissociation rates on the RSV neutralization, particularly
for
     intact antibodies wherein the association rate plays the
     predominant role. Furthermore, we found that antibody binding valence
     also plays a critical role in mediating the viral neutralization through a
     mechanism that is likely unrelated to antibody size or binding avidity.
     We applied an iterative mutagenesis approach, and thereafter were able to
     identify palivizumab Fab variants with up to 1,500-fold
     improvement and palivizumab IgG variants with up to 44-fold
     improvement in the ability to neutralize RSV. These anti-RSV
     immunoprophylaxis. In addition, our findings provide insights into
     engineering potent antibody therapeutics for other disease targets.

L17  ANSWER 2 OF 2     MEDLINE on STN                    DUPLICATE 1
2005293960.    PubMed ID: 15907931.  Ultra-potent antibodies against
     respiratory syncytial virus: effects of binding kinetics and binding
     valence on viral neutralization. Wu Herren; Pfarr David S; Tang Ying; An
     Ling-Ling; Patel Nita K; Watkins Jeffry D; Huse William D; Kiener Peter A;
     Young James F. (MedImmune, Inc., One MedImmune Way, Gaithersburg, MD
     20878, USA.. wuh@medimmune.com) . Journal of molecular biology, (2005 Jul
     1) 350 (1) 126-44.  Journal code: 2985088R. ISSN: 0022-2836. Pub. country:
     England: United Kingdom. Language: English.
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AB We describe here the **selection** of ultra-potent anti-respiratory syncytial virus (RSV) antibodies for preventing RSV infection. A large number of antibody variants derived from Synagis (palivizumab), an anti-RSV monoclonal antibody that targets RSV F protein, were generated by a directed evolution approach that allowed convenient manipulation of the binding kinetics. Palivizumab variants with about 100-fold slower dissociation rates or with fivefold faster **association rates** were identified and tested for their ability to neutralize virus in a microneutralization assay. Our data reveal a major differential effect of the association and dissociation rates on the RSV neutralization, particularly for intact antibodies wherein the **association rate** plays the predominant role. Furthermore, we found that antibody binding valence also plays a critical role in mediating the viral neutralization through a mechanism that is likely unrelated to antibody size or binding avidity. We applied an iterative mutagenesis approach, and thereafter were able to identify palivizumab Fab variants with up to 1500-fold **improvement** and palivizumab IgG variants with up to 44-fold **improvement** in the ability to neutralize RSV. These anti-RSV antibodies likely will offer great clinical potential for RSV immunoprophylaxis. In addition, our findings provide insights into engineering potent antibody therapeutics for other disease targets.

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L18 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2006 ACS on STN
2005:1089103 Ultra-potent antibodies against respiratory syncytial virus:. Wu, H.; Pfarr, D. S.; Tang, Y.; An, L-L.; Pate, N. K.; Watkins, J. D.; Huse, W. D.; Kiener, P. A.; Young, J. F. (USA). Assay and Drug Development Technologies, 3(4), 450-452 (English) 2005. CODEN: ADDTAR. ISSN: 1540-658X. Publisher: Mary Ann Liebert, Inc..
AB We describe there the selection of ultra-potent anti-respiratory syncytial virus (RSV) antibodies for preventing RSV infection.. A large number of antibody variants derived from Synagis (palivizumab), an anti-RSV monoclonal antibody that targets RSV F protein, were generated by a directed evolution approach that allowed convenient manipulation of the binding kinetics. Palivizumab variants with about 100-fold slower dissociation rates or with fivefold faster **association rates** were identified and tested for their ability to neutralize virus in a microneutralization assay. Our data reveal a major differential effect of the association and dissociation rates on the RSV neutralization, particularly for intact antibodies wherein the **association rate** plays the predominant role. Furthermore, we found that antibody binding valence also plays a critical role in mediating the viral neutralization through a mechanism that is likely unrelated to antibody size or binding avidity. We applied an iterative mutagenesis approach, and thereafter were able to identify palivizumab Fab variants with up to 1,500-fold **improvement** and palivizumab IgG variants with up to 44-fold **improvement** in the ability to neutralize RSV. These anti-RSV immunoprophylaxis. In addition, our findings provide insights into engineering potent antibody therapeutics for other disease targets.

L18 ANSWER 2 OF 16 MEDLINE on STN DUPLICATE 1
2005293960. PubMed ID: 15907931. Ultra-potent antibodies against respiratory syncytial virus: effects of binding kinetics and binding valence on viral neutralization. Wu Herren; Pfarr David S; Tang Ying; An Ling-Ling; Patel Nita K; Watkins Jeffry D; Huse William D; Kiener Peter A; Young James F. (MedImmune, Inc., One MedImmune Way, Gaithersburg, MD 20878, USA.. wuh@medimmune.com) . Journal of molecular biology, (2005 Jul

1) 350 (1) 126-44. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB We describe here the selection of ultra-potent anti-respiratory syncytial virus (RSV) antibodies for preventing RSV infection. A large number of antibody variants derived from Synagis (palivizumab), an anti-RSV monoclonal antibody that targets RSV F protein, were generated by a directed evolution approach that allowed convenient manipulation of the binding kinetics. Palivizumab variants with about 100-fold slower dissociation rates or with fivefold faster **association rates** were identified and tested for their ability to neutralize virus in a microneutralization assay. Our data reveal a major differential effect of the association and dissociation rates on the RSV neutralization, particularly for intact antibodies wherein the **association rate** plays the predominant role. Furthermore, we found that antibody binding valence also plays a critical role in mediating the viral neutralization through a mechanism that is likely unrelated to antibody size or binding avidity. We applied an iterative mutagenesis approach, and thereafter were able to identify palivizumab Fab variants with up to 1500-fold **improvement** and palivizumab IgG variants with up to 44-fold **improvement** in the ability to neutralize RSV. These anti-RSV antibodies likely will offer great clinical potential for RSV immunoprophylaxis. In addition, our findings provide insights into engineering potent antibody therapeutics for other disease targets.

L18 ANSWER 3 OF 16 MEDLINE on STN DUPLICATE 2

2004172831. PubMed ID: 14754891. "Network leaning" as a mechanism of insurmountable antagonism of the angiotensin II type 1 receptor by non-peptide antagonists. Takezako Takanobu; Gogonea Camelia; Saad Yasser; Noda Keita; Karnik Sadashiva S. (Department of Molecular Cardiology, Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA.) Journal of biological chemistry, (2004 Apr 9) 279 (15) 15248-57. Electronic Publication: 2004-01-30. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A mechanistic understanding of the insurmountable antagonism of the angiotensin II type 1 (AT(1)) receptor could be fundamental in the quest for discovery and **improvement** of drugs. Candesartan and EXP3174 are competitive, reversible insurmountable antagonists of the AT(1) receptor. They contain di-acidic substitutions, whereas the surmountable antagonist, losartan, contains only one acidic group. We tested the hypothesis that these two classes of ligands interact with the AT(1) receptor through similar but not identical bonds and that the differences in the acid-base group contacts are critical for insurmountable antagonism. By pharmacological characterization of site-directed AT(1) receptor mutants expressed in COS1 cells we show that specific interactions with Gln(257) in transmembrane 6 distinguishes insurmountable antagonists and that abolishing these interactions transforms insurmountable to surmountable antagonism. In the Q257A mutant, the dissociation rate of [(3)H]candesartan is 2.8-fold more than the rate observed with wild type, and the **association rate** was reduced 4-fold lower than the wild type. The pattern of antagonism of angiotensin II concentration-response in the Q257A mutant pretreated with EXP3174 and candesartan is surmountable. We propose that leaning ability of insurmountable antagonists on Gln(257) in the wild-type receptor is the basis of an antagonist-mediated conformational transition, which is responsible for both slow dissociation and inhibition of maximal IP response.

L18 ANSWER 4 OF 16 MEDLINE on STN DUPLICATE 3

2004144036. PubMed ID: 15037082. Fucose depletion from human IgG1 oligosaccharide enhances binding enthalpy and **association rate** between IgG1 and FcgammaRIIIa. Okazaki Akira; Shoji-Hosaka Emi; Nakamura Kazuyasu; Wakitani Masako; Uchida Kazuhisa; Kakita Shingo; Tsumoto Kouhei; Kumagai Izumi; Shitara Kenya. (Tokyo Research Laboratories, Kyowa Hakko Kogyo Co Ltd, 3-6-6 Asahi-machi, Machida-shi,

Tokyo 194-8533, Japan.) Journal of molecular biology, (2004 Mar 5) 336 (5) 1239-49. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB Depletion of fucose from human IgG1 oligosaccharide improves its affinity for Fcgamma receptor IIIa (FcgammaRIIIa). This is the first case where a glycoform modification is shown to improve glycoprotein affinity for the receptors without carbohydrate-binding capacity, suggesting a novel glyco-engineering strategy to improve ligand-receptor binding. To address the mechanisms of affinity **improvement** by the fucose depletion, we used isothermal titration calorimetry (ITC) and biosensor analysis with surface plasmon resonance. ITC demonstrated that IgG1-FcgammaRIIIa binding was driven by favorable binding enthalpy (DeltaH) but opposed by unfavorable binding entropy change (DeltaS). Fucose depletion from IgG1 enhanced the favorable DeltaH, leading to the increase in the binding constant of IgG1 for the receptor by a factor of 20-30. The increase in the affinity was mainly attributed to an enhanced **association rate.** A triple amino acid substitution in IgG1, S298A/E333A/K334A, is also known to improve IgG1 affinity for FcgammaRIIIa. ITC demonstrated that the amino acid substitution attenuated the unfavorable DeltaS resulting in a three- to fourfold increase in the binding constant. The affinity enhancement by the amino acid substitution was due to a reduced dissociation rate. These results indicate that the mechanism of affinity **improvement** by the fucose depletion is quite distinct from that by the amino acid substitution. Defucosylated IgG1 exhibited higher antibody-dependent cellular cytotoxicity (ADCC) than S298A/E333A/K334A-IgG1, showing a correlation between IgG1 affinity for FcgammaRIIIa and ADCC. We also examined the effect of FcgammaRIIIa polymorphism (Val158/Phe158) on IgG1-FcgammaRIIIa binding. The Phe to Val substitution increased FcgammaRIIIa affinity for IgG1 in an enthalpy-driven manner with the reduced dissociation rate. These results together highlight the distinctive functional **improvement** of affinity by IgG1 defucosylation and suggest that engineering of non-interfacial monosaccharides can improve glycoprotein affinity for receptors via an enthalpy-driven and **association rate**-assisted mechanism.

L18 ANSWER 5 OF 16 MEDLINE on STN DUPLICATE 4

2004497170. PubMed ID: 15465055. Substantial energetic **improvement** with minimal structural perturbation in a high affinity mutant antibody. Midelfort K S; Hernandez H H; Lippow S M; Tidor B; Drennan C L; Wittrup K D. (Biological Engineering Division, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.) Journal of molecular biology, (2004 Oct 22) 343 (3) 685-701. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB Here, we compare an antibody with the highest known engineered affinity (K(d)=270 fM) to its high affinity wild-type (K(d)=700 pM) through thermodynamic, kinetic, structural, and theoretical analyses. The 4M5.3 anti-fluorescein single chain antibody fragment (scFv) contains 14 mutations from the wild-type 4-4-20 scFv and has a 1800-fold increase in fluorescein-binding affinity. The dissociation rate is approximately 16,000 times slower in the mutant; however, this substantial **improvement** is offset somewhat by the **association rate**, which is ninefold slower in the mutant. Enthalpic contributions to binding were found by calorimetry to predominate in the differential binding free energy. The crystal structure of the 4M5.3 mutant complexed with antigen was solved to 1.5A resolution and compared with a previously solved structure of an antigen-bound 4-4-20 Fab fragment. Strikingly, the structural comparison shows little difference between the two scFv molecules (backbone RMSD of 0.6A), despite the large difference in affinity. Shape complementarity exhibits a small **improvement** between the variable light chain and variable heavy chain domains within the antibody, but no significant **improvement** in shape complementarity of the antibody with the antigen is observed in the mutant over the wild-type. Theoretical modeling calculations show

electrostatic contributions to binding account for -1.2 kcal/mol to -3.5 kcal/mol of the binding free energy change, of which -1.1 kcal/mol is directly associated with the mutated residue side-chains. The electrostatic analysis reveals several mechanistic explanations for a portion of the **improvement.** Collectively, these data provide an example where very high binding affinity is achieved through the cumulative effect of many small structural alterations.

L18 ANSWER 6 OF 16 MEDLINE on STN DUPLICATE 5
2002249122. PubMed ID: 11988096. Engineering N-terminal domain of tissue inhibitor of metalloproteinase (TIMP)-3 to be a better inhibitor against tumour necrosis factor-alpha-converting enzyme. Lee Meng-Huee; Verma Vandana; Maskos Klaus; Nath Deepa; Knauper Vera; Dodds Philippa; Amour Augustin; Murphy Gillian. (School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.. meng.lee@uea.ac.uk) . Biochemical journal, (2002 May 15) 364 (Pt 1) 227-34. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: England: United Kingdom. Language: English.
AB We previously reported that full-length tissue inhibitor of metalloproteinase-3 (TIMP-3) and its N-terminal domain form (N-TIMP-3) displayed equal binding affinity for tissue necrosis factor-alpha (TNF-alpha)-converting enzyme (TACE). Based on the computer graphic of TACE docked with a TIMP-3 model, we created a number of N-TIMP-3 mutants that showed significant **improvement** in TACE inhibition. Our strategy was to select those N-TIMP-3 residues that were believed to be in actual contact with the active-site pockets of TACE and mutate them to amino acids of a better-fitting nature. The activities of these mutants were examined by measuring their binding affinities (K(app)(i)) and **association rates** (k(on)) against TACE. Nearly all mutants at position Thr-2 exhibited slightly impaired affinity as well as **association rate** constants. On the other hand, some Ser-4 mutants displayed a remarkable increase in their binding tightness with TACE. In fact, the binding affinities of several mutants were less than 60 pM, beyond the sensitivity limits of fluorimetric assays. Further studies on cell-based processing of pro-TNF-alpha demonstrated that wild-type N-TIMP-3 and one of its tight-binding mutants, Ser-4Met, were capable of inhibiting the proteolytic shedding of TNF-alpha. Furthermore, the Ser-4Met mutant was also significantly more active ($P<0.05$) than the wild-type N-TIMP-3 in its cellular inhibition. Comparison of N-TIMP-3 and full-length TIMP-3 revealed that, despite their identical TACE-interaction kinetics, the latter was nearly 10 times more efficient in the inhibition of TNF-alpha shedding, with concomitant implications for the importance of the TIMP-3 C-terminal domain in vivo.

L18 ANSWER 7 OF 16 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
2001:493683 Document No.: PREV200100493683. Electrostatic control of protein-protein docking and electron transfer. Liang, Zhao-Xun [Reprint author]; Nocek, Judith M. [Reprint author]; Mauk, A. Grant; Hoffman, Brian M. [Reprint author]. Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL, 60208, USA. zxliang@chem.nwu.edu. Abstracts of Papers American Chemical Society, (2001) Vol. 222, No. 1-2, pp. BIOL53. print.
Meeting Info.: 222nd National Meeting of the American Chemical Society. Chicago, Illinois, USA. August 26-30, 2001. American Chemical Society.
CODEN: ACSRAL. ISSN: 0065-7727. Language: English.
AB Most of the cell functions are regulated by protein-protein interactions with delicately balanced binding affinity and specificity. Due to the weak affinity and transient nature of the protein complexes involved in many of those protein-protein interactions, both structural and dynamic information are still elusive. In this study, with a model system consists of zinc substituted myoglobin (ZnMb) and cytochrome b5, we demonstrate that the modification of the surface charge of ZnMb by means of chemical modification and site-directed mutagenesis causes profound changes in protein-protein docking specificity and electron transfer (ET) rate. With the help of electrostatic calculations and Brownian dynamic simulations, we show that the **improvement** of the docking

specificity results from the optimization of the electrostatic interactions at the docking interface. This work suggests that the optimal local electrostatic interactions are critical for protein-protein docking to achieve high specificity and modest binding affinity required for fast association/dissociation rate. Meanwhile, this study also suggests that photoinduced electron transfer may be used as a practical tool for studying transient protein-protein interactions.

L18 ANSWER 8 OF 16 CAPLUS COPYRIGHT 2006 ACS on STN
2000:163472 Document No. 133:116938 Improved response of a fluorescence-based metal ion biosensor using engineered carbonic anhydrase variants. Thompson, Richard B.; Zeng, Hui-Hui; Loetz, Michele; McCall, Keith; Fierke, Carol A. (Dep. Biochem. Mol. Biol., Univ. of Maryland, Sch. Med., Baltimore, MD, USA). Proceedings of SPIE-The International Society for Optical Engineering, 3858(Advanced Materials and Optical Systems for Chemical and Biological Detection), 161-166 (English) 1999. CODEN: PSISDG. ISSN: 0277-786X. Publisher: SPIE-The International Society for Optical Engineering.

AB The response time of biosensors which reversibly bind an analyte such as a metal ion is necessarily limited by the kinetics with which the biosensor transducer binds the analyte. In the case of the carbonic anhydrase-based biosensor we have developed the binding kinetics are rather slow, with the wild type human enzyme exhibiting an **association rate** constant ten thousand-fold slower than diffusion-controlled. By designed and combinatorial means the transducer may be mutagenized to achieve nearly diffusion-controlled **association rate** consts., with commensurate **improvement** in response. In addition, a variant of apocarbonic anhydrase was immobilized on quartz, and is shown to response rapidly to changes in free copper ion in the picomolar range.

L18 ANSWER 9 OF 16 MEDLINE on STN DUPLICATE 6
97185711. PubMed ID: 9033392. Contribution of lysine 60f to S1' specificity of thrombin. Rezaie A R; Olson S T. (Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City 73104, USA.) Biochemistry, (1997 Feb 4) 36 (5) 1026-33. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Lys60f has been proposed to limit the S1' substrate binding site specificity of thrombin to small polar P1' residues by occluding the S1' binding pocket, based on the X-ray crystal structure of thrombin. To test this proposal, we prepared a Lys-->Ala (K60fA) mutant of recombinant thrombin and determined whether this mutation enhanced the reactivity of thrombin with a variant inhibitor [antithrombin (AT)-Denver] and a substrate (protein C) containing poorly recognized P1' Leu residues. AT-Denver in the presence of heparin inhibited K60fA thrombin with a second-order **association rate** constant [k = 4.2 +/- 0.1) x 10(5) M-1 s-1] that was 3.2-fold faster than thrombin [k = (1.3 +/- 0.1) x 10(5) M-1 s-1]. Wildtype AT (P1' Ser) under the same conditions inhibited K60fA thrombin with a 2.5-fold slower rate constant [k = (1.1 +/- 0.1) x 10(7) M-1 s-1] than thrombin [k = (2.8 +/- 0.1) x 10(7) M-1 s-1]. These results indicate an overall 8.3-fold **improvement** in the recognition of the P1' Leu of AT-Denver by K60fA thrombin over that of wild-type thrombin; i.e., the K60fA mutation partly overcomes the defect in thrombin inhibition produced by the P1' mutation in AT-Denver. Resolution of the two-step reactions of AT and AT-Denver with wild-type and mutant thrombins revealed that the enhanced recognition of P1' Leu in AT-Denver by K60fA thrombin occurs primarily in the second reaction step in which a noncovalent AT-thrombin encounter complex is converted to a stable, covalent complex. Thrombin K60fA activated Gla-domainless protein C (GDPC) approximately 2- and approximately 4-fold faster than thrombin in the presence and absence of thrombomodulin (TM), respectively, consistent with an improved interaction of the Leu P1' residue with the mutant S1' pocket. In contrast, the mutant thrombin clotted fibrinogen (P1' Gly) approximately 3-fold slower than thrombin. Kinetic analysis revealed that the **improvement** in the catalytic rate of activation of GDPC by K60fA thrombin in the presence of TM was localized in the second reaction

step, as reflected by an approximately 2-fold increase in kcat. Direct binding studies showed that the K60fA mutation minimally affected the affinity of thrombin for Na+, indicating that the changes in S1' site-specificity of K60fA thrombin did not result from altering the allosteric transition induced by Na+. We conclude that Lys60f limits the P1' substrate and inhibitor specificity of thrombin by influencing the size and polarity of the S1' site which thereby affects the stability of the transition state for cleavage of the scissile bond in the second reaction step.

L18 ANSWER 10 OF 16 MEDLINE on STN DUPLICATE 7
97110321. PubMed ID: 8952482. Efficient **improvement** of hammerhead ribozyme mediated cleavage of long substrates by oligonucleotide facilitators. Jankowsky E; Schwenzer B. (Institut fur Biochemie, Technische Universitat Dresden, Germany.) Biochemistry, (1996 Dec 3) 35 (48) 15313-21. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Hammerhead ribozymes were found to be not very efficient in cleaving long RNA substrates in trans. Oligonucleotide facilitators, capable of affecting hammerhead ribozymes by interacting with the substrate at the termini of the ribozyme, may improve this reaction. We determined in vitro the effects of 18 DNA and RNA oligonucleotide facilitators on three substrates containing 39, 452, and 942 nucleotides, respectively, by estimating the facilitator influences on association between ribozyme and substrate and on the cleavage step. The effects increase with the length of the substrates. With the 39mer substrate a maximal 4-fold enhancement of the ribozyme activity could be detected, the reaction with the 942mer substrate was accelerated up to 115-fold by facilitator addition. In long, structured substrates the facilitators have the potential to preform the substrate for the ribozyme attack. Due to this preforming effect, the rate of ribozyme-substrate association was increased as well as the rate of the cleavage step. 3'-End facilitators accelerate both of these rates, largely independent on the facilitator length. The rate of the cleavage step is raised as a result of a favorable activation energy gain by these facilitators. With all substrates, the 5'-end facilitators increase the **association rate** between ribozyme and substrate in dependence on their length. With the 39mer substrate the 5'-end facilitators decrease the rate of the cleavage step. With the long substrates 5'-end facilitators partially increase the rate of the cleavage step due to their preforming potential with these substrates. In some examples, combinations of several 5'-end and 3'-end facilitators provide an additional **improvement** over single facilitators in both the association between ribozyme and substrate and the cleavage step. Results suggest that even short facilitators may be efficient effectors enhancing hammerhead ribozyme mediated cleavage of long substrates.

L18 ANSWER 11 OF 16 MEDLINE on STN DUPLICATE 8
95263530. PubMed ID: 7744836. Development of a novel recombinant serpin with potential antithrombotic properties. Hopkins P C; Crowther D C; Carrell R W; Stone S R. (Department of Haematology, University of Cambridge, United Kingdom.) Journal of biological chemistry, (1995 May 19) 270 (20) 11866-71. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Recombinant alpha 1-antitrypsin with a P1 arginine residue (Arg-alpha 1-antitrypsin) is a rapid inhibitor of both thrombin and activated protein C (APC). A series of mutants were made in an attempt to increase the specificity of this serpin for thrombin over APC. Initially, P2 and P'1 residues of Arg-alpha 1-antitrypsin were replaced in single and double mutations by the corresponding residues in antithrombin and C1 inhibitor which are very poor inhibitors of APC. No **improvement** in selectivity was achieved by these mutations. In fact, all P2/P'1 substitutions led to a decrease in selectivity for thrombin over APC. For example, replacement of the P2 proline of Arg-alpha 1-antitrypsin by glycine decreased the **association rate** constant (kass) with thrombin by 37-fold while the kass value with APC was reduced by only

16-fold. Cooperative effects were observed with the double P2 and P'1 substitutions; the mutational effects were not additive. The decrease in the kass for thrombin caused by the mutation of the P2 proline to alanine or glycine was 3-fold greater when threonine was present in the P'1 position instead of the normal serine. In contrast to the disappointing results with the P2/P'1 mutations, replacement of the P7 to P'3 residues of alpha 1-antitrypsin by those of antithrombin led to a dramatic increase in selectivity. Although this substitution only affected the kass value with thrombin by 10-fold, a 12,500-fold decrease in this value with APC was observed. Substitution of proline for the P2 glycine of this chimeric serpin increased the kass values with thrombin and APC by 7- and 90-fold, respectively. The effect of the P2 substitution was again found to depend on the sequence surrounding the residue; the change in the kass for APC caused by the P2 Pro-->Gly replacement was 6-fold larger in the chimeric serpin. Evaluation of the kass values of the chimeric serpin with a P2 proline in light of the likely rates of inhibition of thrombin and APC during antithrombotic therapy with heparin suggested that this serpin may have kinetic parameters suitable for an antithrombotic agent.

L18 ANSWER 12 OF 16 MEDLINE on STN DUPLICATE 9

95286993. PubMed ID: 7769242. Characterization of the interaction of alkaline phosphatase with an activity inhibiting monoclonal antibody by progress curve analysis. Cumme G A; Walter U; Bublitz R; Hoppe H; Rhode H; Horn A. (Institute of Biochemistry, Friedrich Schiller University, Jena, Germany.) Journal of immunological methods, (1995 May 11) 182 (1) 29-39. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Using the enzyme activity inhibiting monoclonal antibody IB 10B8 against alkaline phosphatase of calf intestine (AP), the interaction of a macromolecular antigen with the antibody was studied with different reaction conditions and with different conformations of the antigen, i.e. using (i) different pH values, (ii) different temperatures, (iii) different substrate saturation of the enzyme, (iv) different glycosylphosphatidyl-AP (GPI-AP) aggregates, and (v) membrane-bound species. In the case of antibody excess and negligible substrate consumption enzymic product formation proceeds according to [P] = a + b x t - c x exp(-d x t). By direct progress curve fitting and secondary data evaluation using nonlinear regression, omitting numerical derivation and graphic techniques, kinetic constants of the immune reaction have been estimated. The method does not require any artificial labelling nor any separation of bound and free entities. (i) Upon increasing pH from 9.8 to 11.0, the dissociation constant of the enzyme-antibody complex is increased strongly, mainly due to the decreasing **association rate** constant. (ii) A temperature increase from 25 degrees C to 37 degrees C produces a marked increase of both the association and dissociation rate constant. (iii) To differentiate between the interaction of the antibody with the free (E) and substrate-bound (ES) enzyme, experiments were done at different substrate concentrations. The results were fitted to a model allowing determination of association and dissociation rate constants of the free and substrate-bound enzyme. The inverse variation of association and dissociation rate constants caused by substrate binding produces a marked increase of the dissociation constant of the antibody-enzyme complex. The antibody-bound enzyme shows a nearly three-fold higher Km value and a six-fold lower catalytic constant as compared to the free enzyme. (iv) Investigations of the interaction of the antibody with anchorless AP, different hydrophobic aggregates of purified GPI-AP (fractions II-V). (v) Membrane-bound GPI-AP show that the epitopes of all species are fully accessible to the antibody and not cryptic. Surprisingly the insertion of the GPI-moiety into the membrane and the aggregation of the different GPI-AP fractions II-V seem to improve antibody binding. Such **improvement** of binding was not found in control experiments with Fab, indicating only for the bivalent antibody a stronger interaction with the multivalent antigen than with the monovalent antigen.

L18 ANSWER 13 OF 16 MEDLINE on STN DUPLICATE 10
92028940. PubMed ID: 1930217. Calcium binding to fluorescent calcium indicators: calcium green, calcium orange and calcium crimson. Eberhard M; Erne P. (Department of Research, Kantonsspital, Basel, Switzerland.) Biochemical and biophysical research communications, (1991 Oct 15) 180 (1) 209-15. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB The recently introduced fluorescent calcium sensitive indicators calcium green, calcium orange and calcium crimson suggest important **improvements** and advantages to detect small calcium transients at low indicator concentrations. Thermodynamic dissociation constants and dissociation rate constants of calcium green, calcium orange and calcium crimson were measured by use of fluorescence titration and stopped flow fluorescence, respectively. Calcium binding to the indicators conforms to a 1:1 calcium:indicator complex although at high concentrations of calcium the fluorescence properties deviate somewhat from the behaviour predicted by the simple model. Dissociation of the calcium-indicator complex was found to be monoexponential under all conditions examined. The affinity for calcium of the three indicators generally increases with raising temperatures (Kd at 11.5 degrees C and 39.7 degrees C (nM): 261, 180 for calcium green; 527, 323 for calcium orange; 261, 204 for calcium crimson) and pH (Kd at pH 6.42 and 7.40 (nM): 314, 226 for calcium green; 562, 457 for calcium orange; 571, 269 for calcium crimson). The changes of the thermodynamic dissociation constant are mainly caused by changes of the **association rate** constant. The temperature dependence of calcium binding to the indicators revealed that this process is entropically favoured at ambient temperature.

L18 ANSWER 14 OF 16 MEDLINE on STN DUPLICATE 11
90042304. PubMed ID: 2811365. **Improvement** in glucocorticoid receptor binding affinity concomitant to shift from antagonist to agonist activity in a series of 17 beta-carboxamide derivatives of dexamethasone. Lefebvre P; Formstecher P; Rousseau G G; Lustenberger P; Dautrevaux M. (Laboratoire de Biochimie Structurale, Faculte de Medecine, Lille, France.) Journal of steroid biochemistry, (1989 Oct) 33 (4A) 557-63. Journal code: 0260125. ISSN: 0022-4731. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Modification of the 17 beta-side chain of the synthetic glucocorticoid agonist dexamethasone by periodic oxidation and subsequent coupling to various primary amines yield secondary 17 beta-carboxamide derivatives displaying antiglucocorticoid activity in vitro, but not in vivo. To obtain more potent antiglucocorticoids, new secondary and tertiary 17 beta-carboxamide derivatives were synthesized. Although they displayed an improved affinity for the glucocorticoid receptor in rat thymus cytosol and antiglucocorticoid activity in rat hepatoma (HTC) cells, these new compounds were again devoid of in vivo antiglucocorticoid activity in the rat. Moreover, the increase in receptor binding affinity was correlated for most compounds with the appearance of a partial agonist activity in HTC cells. The tertiary 17 beta-carboxamide derivative DX diMe displayed the highest affinity but was also a partial agonist in vivo. Kinetic studies with several tritiated 17 beta-carboxamide derivatives showed that they had **association rate** constants similar to that of dexamethasone, but different dissociation rate constants. The rapid dissociation of the compounds displaying antiglucocorticoid activity contrasted with the slow dissociation of DX diMe. Therefore, antiglucocorticoid activity in the 17 beta-carboxamide series is probably related to the formation of rapidly dissociating glucocorticoid receptor-ligand complexes that are unable to undergo the transformation step.

L18 ANSWER 15 OF 16 CAPLUS COPYRIGHT 2006 ACS on STN
1988:158573 Document No. 108:158573 Search for helium hydride cation (HeH+) in NGC 7027. Moorhead, J. M.; Lowe, R. P.; Maillard, J. P.; Wehlau, W. H.; Bernath, P. F. (Univ. West. Ontario, London, ON, N6A 3K7, Can.). Astrophysical Journal, 326(2, Pt. 1), 899-904 (English) 1988. CODEN:

ASJOAB. ISSN: 0004-637X.
AB The 3.3 μm spectrum was studied of the planetary nebula NGC 7027 for the R(0) line of the fundamental vibration-rotation band of the mol. ion HeH+ without detecting it. The upper limit for detection is 3.7 + 10-14 ergs cm-2 s-1, an **improvement** in excess of 100 over previous published attempts. This limit is low when compared to expectations based on anal. of mol. processes in gaseous nebula. Likely causes are incorrect radiative **association rates** or inadequate representation of the nebular size or of the d. distribution in the outer boundary of the nebular model used for the flux prediction.

L18 ANSWER 16 OF 16 CAPLUS COPYRIGHT 2006 ACS on STN
1939:56370 Document No. 33:56370 Original Reference No. 33:8091f-g Some remarks on the theory of reaction rates. Wigner, Eugene P. Journal of Chemical Physics, 7, 646-52 (Unavailable) 1939. CODEN: JCPSA6. ISSN: 0021-9606.
AB Effects connected with a more complicated nature of the energy surfaces of relatively simple reactions are discussed. Better results are obtained in calculating the rate of association of atoms when 3 addnl. attraction states are considered. The calculated and exptl. rates of association of I, Br and Cl atoms are compared. The **improvement** expected from consideration of the angular momentum of the associating pair of atoms is discussed. Discussion by others is included.

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L1        28669 S ASSOCIATION CONSTANT
L2            5 S L1 AND THERAPEUTIC POTENTIAL
L3            2 DUP REMOVE L2 (3 DUPLICATES REMOVED)
L4          242 S L1 AND (8 X 10)
L5            1 S L4 AND IMPROVE
L6            2 S L1 AND MODIFIED PEPTIDE
L7            2 DUP REMOVE L6 (0 DUPLICATES REMOVED)
L8            0 S METHOD OPTIMIZING THERAPEUTIC POTENCY
L9            0 S OPTIMIZING THERAPEUTIC POTENCY
L10         795 S THERAPEUTIC POTENCY
L11          81 S L10 AND IMPROVE
L12           0 S L11 AND AMINO ACID SUBSTITUTION
L13           1 S L10 AND ASSOCIATION RATE
L14        9828 S ASSOCIATION RATE
L15          56 S L14 AND IMPROVEMENT
L16           6 S L15 AND SELECTION
L17           2 DUP REMOVE L16 (4 DUPLICATES REMOVED)
L18          16 DUP REMOVE L15 (40 DUPLICATES REMOVED)

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L19          31 L1 AND 4 FOLD INCREASE

=> dup remove l19
PROCESSING COMPLETED FOR L19
L20          14 DUP REMOVE L19 (17 DUPLICATES REMOVED)

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L20 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
1998:333147 Document No. 129:149061 Electrochemical and binding properties of a novel ferrocene-containing redox-active basket-shaped host molecule. Dol, Georg C.; Kamer, Paul C. J.; Hartl, Frantisek; van Leeuwen, Piet W.
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N. M.; Nolte, Roeland J. M. (J. H. van't Hoff Research Institute, University of Amsterdam, Amsterdam, 1018 WV, Neth.). Journal of the Chemical Society, Dalton Transactions: Inorganic Chemistry (12), 2083-2090 (English) 1998. CODEN: JCDTBI. ISSN: 0300-9246. OTHER SOURCES: CASREACT 129:149061. Publisher: Royal Society of Chemistry.

AB A novel basket-shaped host based on the rigid mol. diphenylglycoluril {3a,6a-diphenyltetrahydroimidazo[4,5-d]imidazole-2,5(1H,3H)-dione} was synthesized and characterized. It is able to bind cations in its crown ether rings and neutral organic substrates in its cavity. Differential pulse voltammetry expts. showed that the host is redox-responsive to cations. It forms 1:1 complexes with K+ and NH4+ ions and 1:2 host-guest complexes with Na+ ions. On addition of (di)ammonium salts, protonation of the host occurs. A complex was formed between the host and model substrate olivetol (5-pentylbenzene-1,3-diol). In the absence of additives, this complex is stabilized via H bonds and π-π stacking interactions. In the presence of Na+ ions a complex consisting of the host, the diol, and 2 Na+ ions was formed, in which H bonds are no longer present. In the presence of 2 Na+ ions a **4-fold increase** in **association constant** was found. Spectroscopic (NMR and IR) expts. were used to elucidate the mode of cooperative coordination between the host, diol substrate and Na+ ions.

L20 ANSWER 2 OF 14 MEDLINE on STN DUPLICATE 1

95204435. PubMed ID: 7896784. Sequence-specific DNA recognition by the SmaI endonuclease. Withers B E; Dunbar J C. (Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan 48201.) Journal of biological chemistry, (1995 Mar 24) 270 (12) 6496-504. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB SmaI endonuclease recognizes and cleaves the sequence CCC decreases GGG. The enzyme requires magnesium for catalysis; however, equilibrium binding assays revealed that the enzyme binds specifically to DNA in the absence of magnesium. A specific **association constant** of 0.9 x 10(8) M-1 was determined for SmaI binding to a 22-base duplex oligonucleotide. Furthermore, the KA was a function of the length of the DNA substrate and the enzyme exhibited an affinity of 1.2 x 10(9) M-1 for a 195-base pair fragment and which represented a 10(4)-**fold increase** in affinity over binding to nonspecific sequences. A Km of 17.5 nM was estimated from kinetic assays based on cleavage of the 22-base oligonucleotide and is not significantly different from the KD estimated from the thermodynamic analyses. Footprinting (dimethyl sulfate and missing nucleoside) analyses revealed that SmaI interacts with each of the base pairs within the recognition sequence. Ethylation interference assays suggested that the protein contacts three adjacent phosphates on each strand of the recognition sequence. Significantly, a predicted protein contact with the phosphate 3' of the scissile bond may have implications in the mechanism of catalysis by SmaI.

L20 ANSWER 3 OF 14 MEDLINE on STN DUPLICATE 2

96032853. PubMed ID: 7567471. DNA determinants in sequence-specific recognition by XmaI endonuclease. Withers B E; Dunbar J C. (Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201, USA.) Nucleic acids research, (1995 Sep 11) 23 (17) 3571-7. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The XmaI endonuclease recognizes and cleaves the sequence C decreases CCGGG. Magnesium is required for catalysis, however, the enzyme forms stable, specific complexes with DNA in the absence of magnesium. An **association constant** of 1.2 x 10(9)/M was estimated for the affinity of the enzyme for a specific 195 bp fragment. Competition assays revealed that the site-specific **association constant** represented an approximately 10(4)-**fold increase** in affinity over that for non-cognate sites. Missing nucleoside analyses suggested an interaction of the enzyme with each of the cytosines and guanines within the recognition site. Recognition of

each of the guanines was also indicated by dimethylsulfate interference footprinting assays. The phosphates 5' to the guanines within the recognition site appeared to be the major sites of interaction of XmaI with the sugar-phosphate backbone. No significant interaction of the protein was observed with phosphates flanking the recognition sequence. Comparison of the footprinting patterns of XmaI with those of the neoschizomer SmaI (CCC decreases GGG) revealed that the two enzymes utilize the same DNA determinants in their specific interaction with the CCCGGG recognition site.

L20 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN
1996:398596 Document No. 125:80240 Dehydration of interacting protein surfaces and the stability of a protein-protein complex: A titration calorimetry study. Jelesarov, Ilian; Bosshard, Hans Rudolf (Department Biochemistry, University Zurich, Zurich, CH-8057, Switz.). Perspectives on Protein Engineering & Complementary Technologies, Collected Papers, International Symposium, 3rd, Oxford, Sept. 13-17, 1994, Meeting Date 1994, 273-274. Editor(s): Geisow, Michael J.; Epton, Roger. Mayflower Worldwide: Kingswinford, UK. (English) 1995. CODEN: 62ZQAP.
AB The association of spinach ferredoxin (Fd) with ferredoxin:NADP+ reductase (FNR) was characterized by isothermal titration calorimetry (ITC). In the physiol. temperature range, formation of the complex is mainly driven by a pos. entropy change. Binding is accompanied by a moderate neg. heat capacity change, ΔCp. The favorable binding entropy and neg. ΔCp indicate large contribution from hydrophobic effects. Dehydration of the protein-protein interface was demonstrated by osmotic stress expts. The **4-fold increase** of the **association constant** in the presence of 52% glycerol points to the importance of water release to the stability of the complex.

L20 ANSWER 5 OF 14 MEDLINE on STN DUPLICATE 3
91329333. PubMed ID: 1868050. Self-association of human and porcine relaxin as assessed by analytical ultracentrifugation and circular dichroism. Shire S J; Holladay L A; Rinderknecht E. (Department of Pharmaceutical Research and Development, Genentech, Inc., South San Francisco, California 94080.) Biochemistry, (1991 Aug 6) 30 (31) 7703-11. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.
AB The self-association properties of recombinant DNA derived human relaxin, and porcine relaxin isolated from porcine ovaries, have been studied by sedimentation equilibrium analytical ultracentrifugation and circular dichroism (CD). The human relaxin ultracentrifuge data were adequately defined by a monomer-dimer self-association model with an **association constant** of approximately 6 x 10(5) M-1, whereas porcine relaxin was essentially monomeric in solution. An approximate 5-fold increase in weight fraction of human relaxin monomer elicited by dilution of the protein resulted in no change in the far-UV CD spectrum at 220 nm. In contrast, after the same increase in weight fraction of monomer, the near-UV circular dichroism spectra for human relaxin exhibited a significant decrease in the amplitude for the CD bands near 277 and 284 nm. These CD bands, which may be assigned to the lone tyrosine in human relaxin, are superimposed on a broad envelope that is probably due to the three disulfide chromophores. Although both the human and porcine proteins contain two tryptophan residues, the near-UV CD spectra exhibit only a broad shoulder near 295 nm rather than the strong CD bands often found for tryptophan. Moreover, there is little change in this broad band after dilution of human relaxin to concentrations that resulted in a **4-fold increase** in monomer weight fraction. These data suggest that dissociation of the human relaxin dimer to monomer is not accompanied by large overall changes in secondary structure or alteration in the average tryptophan environment, whereas there is a significant change in the tyrosine environment. (ABSTRACT TRUNCATED AT 250 WORDS)

L20 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN

1991:623989 Document No. 115:223989 Evidence that androgen-binding protein endocytosis in vitro is receptor mediated in principal cells of the rat epididymis. Gueant, J. L.; Fremont, S.; Felden, F.; Nicolas, J. P.; Gerard, A.; Leheup, B.; Gerard, H.; Grignon, G. (Fac. Med., Univ. Nancy I, Vandoeuvre-les-Nancy, 54505, Fr.). Journal of Molecular Endocrinology, 7(2), 113-22 (English) 1991. CODEN: JMLEEI. ISSN: 0952-5041.

AB The binding of [125I-iodo]androgen-binding protein (ABP) and of [3H]Δ6-testosterone photoaffinity-labeled ABP to receptors in the plasma membrane of rat epididymal cells were studied in 3 ways: ABP binding to a Triton X-100-solubilized membrane extract, ABP binding to isolated epithelial cells in suspension, and autoradiog. of segments of dissected epididymides after in-vitro intraluminal injection of labeled ABP. The binding of iodinated ABP to the receptor was similar to that of photoaffinity-labeled ABP in gel filtration. The ABP-receptor complex was eluted from Superose 6 gels as an aggregate, with a mol. mass of 2000 kDa. It was separated into 2 peaks by sucrose gradient ultracentrifugation, with resp. sedimentation coeffs. of 18.4 and 9.0 S. The activity of the receptor (ABP-binding capacity/mg protein) was 10-fold higher in the caput than in the cauda. The binding of ABP to the receptor was pH dependent, being almost abolished at pH <4. The binding at 4° of photoaffinity-labeled ABP to epithelial cells corresponded to 2 types of binding sites. The nos. of high-affinity and low-affinity sites per cell were 1600 and 7700, resp.; the **association consts.** of these sites were 67.9 and 2.8 L/nM, resp. The binding was decreased by treatment of the cells with trypsin or incubation in the presence of EDTA. The binding in vitro of labeled ABP to the epididymis epithelium reached a maximum after about 20 min at 4°. In the autoradiog. study the tracer was closely associated with coated pits, coated vesicles, endosomes and pale multivesicular bodies. Treatment of rats with cycloheximide reduced the uptake of the tracer. Perfusion in vitro of epididymides with chloroquine produced a **4-fold increase** of the tracer in endosomes and multivesicular bodies.

L20 ANSWER 7 OF 14 MEDLINE on STN DUPLICATE 4

89306123. PubMed ID: 2744216. Nuclear acceptor sites for estrogen-receptor complexes in the liver of the turtle, Chrysemys picta. I. Sexual differences, species specificity and hormonal dependency. Yu M S; Ho S M. (Department of Biology, Tufts University, Medford, MA 02155.) Molecular and cellular endocrinology, (1989 Jan) 61 (1) 37-48. Journal code: 7500844. ISSN: 0303-7207. Pub. country: Netherlands. Language: English.

AB Hepatic estrogen receptors (ERs) of the female turtle, Chrysemys picta, when complexed with [3H]estradiol ([3H]E2), were shown to bind specifically to liver chromatin isolated from the same species. The binding of the [3H]E2 receptor complex to chromatin requires both the steroid ligand and the receptor protein. Maximal binding occurred within 60-70 min of incubation at 4 degrees C in a Tris buffer containing 0.1 M KCl. The binding of the [3H]E2 receptor complex to intact chromatin was saturable, whereas the binding to turtle or calf thymus DNA remained linear. Scatchard analyses revealed more estrogen receptor binding sites on hepatic chromatin isolated from female turtles than that prepared from the males (binding capacities: female chromatin = 67.9 +/- 6.8 fmol/mg DNA equivalent; male chromatin = 28.5 +/- 2.5 fmol/mg DNA equivalent). Furthermore, the [3H]E2 receptor complex was bound with a higher affinity to female chromatin than to male chromatin (**association constants**: female chromatin = 11.7 +/- 2.7 X 10(10) M-1; male chromatin = 2.5 +/- 0.7 X 10(10) M-1). In contrast to turtle hepatic [3H]E2 receptors, ERs in rat liver or mouse uterine cytosol exhibited little binding affinity for hepatic chromatin isolated from the turtle. Tissue specificity was demonstrated in the interaction of the [3H]E2 receptor complex and chromatin; high affinity, saturable binding of the [3H]E2 receptor complex was only observed on chromatin isolated from the liver but not on those prepared from the heart, kidney and muscle. A 3- to **4-fold increase** in the number of hepatic chromatin [3H]E2 receptor binding sites was observed in 21-day ovariectomized or hypophysectomized female (capacities = 209.3 +/- 6.1 and

270 +/- 10.1 fmol/mg DNA equivalent, respectively). It is postulated that [3H]E2 receptor binding sites on the chromatin of intact females are partially 'masked', and removal of a gonadal and/or pituitary factor(s) unveils additional binding sites on the female chromatin. This paper is first to report the presence of high affinity, species- and tissue-specific acceptor sites on the liver chromatin of a reptilian species. The fact that the levels and properties of these acceptor sites are dependent on the sex and hormonal state of the animal suggests that they may play a role in the regulation of hepatic estrogen responsiveness and vitellogenesis in this species.

L20 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2006 ACS on STN

1988:106640 Document No. 108:106640 Study of the role of histidyl, tyrosyl, α- or ε-amino residues in the specific binding of 3,5,3'-triiodothyronine to rat liver nuclear receptors. Brtko, Julius; Knopp, Jan (Inst. Exp. Endocrinol., Cent. Physiol. Sci., Bratislava, Czech.). Acta Endocrinologica, 117(2), 159-65 (English) 1988. CODEN: ACENA7. ISSN: 0001-5598.

AB The role of histidyl, tyrosyl, α-or ε-amino residues of rat liver nuclear receptors for the specific binding of T3 was studied by chemical modifying the receptor mol. The kinetics of the formation of N-carbethoxyhistidyl derivative from histidyl groups of nuclear receptors by diethylpyrocarbonate was examined The modified nuclear receptor fraction was separated from diethylpyrocarbonate by gel filtration and the T3 binding parameters equilibrium **association constant** (Ka) and maximum binding capacity (MBC) at pH 8.0 were tested by Scatchard plot anal. At 0.1 mmol/L diethylpyrocarbonate, the value of Ka was decreased without any change in MBC. The modification of α- or ε-amino groups of nuclear receptors by excess of trinitrobenzenesulfonic acid, 6.3 mmol/L at pH 8.5, resulted in a **4-fold increase** in MBC of T3 specific binding without any change in Ka. In addition, acetylation of tyrosyl residues of nuclear receptors at pH 7.5 with an excess of 24 mmol/L N-acetylimidazole was performed. No changes in nuclear receptor Ka or MBC were observed after N-acetylimidazole treatment. Histidine and/or amino groups of the receptor mol. seem to hold a key position in the generation of the biol. active T3-nuclear receptor complex in the rat liver.

L20 ANSWER 9 OF 14 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 5

1983:159886 Document No.: PREV198375009886; BA75:9886. ACTIVATION OF RABBIT MUSCLE LACTATE DEHYDROGENASE BY PHOSPHATE ACTIVE ENZYME GEL CHROMATOGRAPHY AND ENZYME KINETIC STUDIES. WARD L D [Reprint author]; WINZOR D J. DEP BIOCHEMISTRY, UNIV QUEENSLAND, ST LUCIA, QUEENSLAND 4067, AUSTRALIA. Archives of Biochemistry and Biophysics, (1982) Vol. 216, No. 1, pp. 329-336.
CODEN: ABBIA4. ISSN: 0003-9861. Language: ENGLISH.

AB Enzyme kinetic studies are presented which demonstrate the activating effect of phosphate on the conversion of pyruvate to lactate by rabbit muscle lactate dehydrogenase. A simple method of active enzyme gel chromatography is used to preclude the possibility that this effect is due to redistribution of enzyme between tetrameric and dissociated states as the result of preferential binding of phosphate to the tetrameric enzymatic form. By analysis of the kinetic results in terms of an ordered 2-substrate mechanism, the source of the activation is traced to enhancement of the strength of the enzyme-NADH interaction, primarily because in the rate constant for the formation of the binary enzyme-coenzyme complex. Preliminary estimates of the relevant equilibrium constants from the kinetic data indicate that the binding of phosphate to rabbit muscle lactate dehydrogenase leads to 2- to **4-fold increase** in the intrinsic **association constant** for the interaction between NADH and the enzyme under the conditions (pH 7.4, I [ionic, strength] = 0.15) used to study the activation phenomenon.

L20 ANSWER 10 OF 14 MEDLINE on STN DUPLICATE 6

81232851. PubMed ID: 7248272. Fluorescent substrate analogue for adenosine deaminase: 3'-O-[5-(dimethylamino)naphthalene-1-sulfonyl]adenosine. Skorka G; Shuker P; Gill D; Zabicky J; Parola A H. Biochemistry, (1981 May 26) 20 (11) 3103-9. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The synthesis of the fluorescent derivative of adenosine, by reaction with 5-(dimethylamino)naphthalene-1-sulfonyl chloride in dry pyridine at low temperature, yielding 3'-O-[5-(dimethylamino)naphthalene-1-sulfonyl]adenosine (3'-O-dansyladenosine), is here described. 3'-O-Dansyladenosine is partially soluble in water (approximately 10(-4) M) and upon excitation at 325 nm exhibits maximum fluorescence emission at 516 +/- 22 nm (corrected) in buffered aqueous solution at pH 7.6 with a quantum yield of 0.21 and a lifetime of 11.8 +/- 0.2 ns. The fluorescence of 3'-O-dansyladenosine is sensitive to the polarity of its solvent: in pyridine, a quantum yield of 0.61 at the emission maximum of 435 nm was observed. 3'-O-Dansyladenosine is a reversible competitive inhibitor of adenosine deaminase with a moderate inhibitive dissociation constant K1 = (1.54 +/- 0.13) X 10(-5) M. The enzyme-substrate analogue **association constant** was determined by equilibrium dialysis to be K = (0.69 +/- 0.05) X 10(5) M-1, very close to KI-1. The hydrophobic nature of its binding site in adenosine deaminase is evident from the strong blue shift of the fluorescence emission maximum to 440 nm, the **4-fold increase** in fluorescence quantum yield, and the longer lifetime of 15.8 +/- 0.2 ns; the tight, rigid nature of the complex is evident from its high fluorescence polarization value, 0.23. An 85% decrease in the fluorescence emission intensity of the adenosine deaminase-3'-O-dansyladenosine complex in the presence of adenosine indicates the selective binding to the enzyme active site. Correlation between the conformation of the probe, either when free in various solvents or when bound to the enzyme, and its fluorescence quantum yield is noted. 3'-O-Dansyladenosine is suitable for fluorescent labeling of adenosine deaminase in cell systems.

L20 ANSWER 11 OF 14 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 7

81110485 EMBASE Document No.: 1981110485. Regulation of pituitary gonadotropin-releasing hormone receptors by gonadal hormones. Clayton R.N.; Catt K.J.. Endocrinol. Reproduct. Res. Branch, Nat. Inst. Child Hlth Hum. Developm., NIH, Bethesda, Md. 20205, United States. Endocrinology Vol. 108, No. 3, pp. 887-895 1981.
CODEN: ENDOAO
Pub. Country: United States. Language: English.

ED Entered STN: 911209

AB The regulatory role of pituitary gonadotropin-releasing hormone (GnRH) receptors in the control of gonadotropin secretion was investigated in male and female rats after castration and sex steroid hormone replacement. GnRH receptors were measured in homogenates of individual pituitaries by equilibration with 125I-labeled [D-Ser(tBu)6]des-Gly10-GnRh N-ethylamide, and compared with serum and pituitary LH concentrations. The equilibrium **association constants** (K(a)) were 6.1 and 5.1 x 109 M-1 for intact and castrate male rat pituitaries, respectively. After orchidectomy, pituitary GnRH receptor concentration increased by 75% at 24 h, from 150 fmol to 250 fmol/gland, while serum LH levels increased 10-fold (30 to 300 ng/ml). There was a further slight increase in the GnRH receptor concentration (to 370 fmol/gland) and serum LH (to 500 ng/ml) over the ensuing 10 days, and at 15 and 20 days GnRH receptors were 304 and 306 fmol/gland, respectively. There was a highly significant ($P < 0.001$) positive correlation between basal serum LH and FSH concentrations and the pituitary GnRH receptor content measured in individual animals. Treatment with testosterone propionate (100 μg/day) completely prevented the GnRH receptor and serum LH responses to castration, while 50 μg testosterone propionate/day produced variable results. 17β-Estradiol (5 μg/day), diethylstilbestrol (5 μg/day), and dihydrotestosterone (50 μg/day) prevented the increase in GnRH

receptors 5 days after orchidectomy, while serum LH levels were only partially suppressed. In adult female rats, ovariectomy caused a 2- to **4-fold increase** in serum LH in the first 3 days, followed by a larger secondary increase of 10- to 15-fold after 5 days. GnRH receptor concentration increased from 130 to 240 fmol/gland on the third day after operation, just before the major rise in serum LH. 17β-Estradiol (1 μg/day), progesterone (2.5 mg/day), and estradiol plus progesterone inhibited the postovariectomy rise in GnRH receptors for up to 11 days. Estradiol or progesterone given alone prevented the initial rise in serum LH but not the secondary rise from 5 days on, while the combination of estradiol plus progesterone was effective in this regard for the entire treatment period. These results indicate that increased pituitary binding of GnRH is a significant component of the mechanism responsible for postcastration elevations of gonadotropin secretion.

L20 ANSWER 12 OF 14 EMBASE DUPLICATE 8

81212303 EMBASE Document No.: 1981212303. Effect of the structure-stabilizing agent glycerol on detergent-solubilized follicle-stimulating hormone receptors from calf testis. Dias J.A.; Huston J.S.; Reichert Jr. L.E.. Dept. Biochem., Albany Med. Coll., Union Univ., Albany, NY 12208, United States. Endocrinology Vol. 109, No. 3, pp. 736-742 1981.
CODEN: ENDOAO
Pub. Country: United States. Language: English.

ED Entered STN: 911209

AB The nonionic detergent Triton X-100 is widely used to solubilize integral membrane proteins, such as peptide hormone receptors. Solubilization of the FSH receptor from calf testes membrane with Triton X-100 is accompanied by a complete loss of [125I]idodohuman FSH ([125I]iodo-hFSH)-binding activity after 24 h at 4 C, presumably as a consequence of its structural destabilization. In the presence of Triton X-100 and the structure-stabilizing agent glycerol, however, [125I]iodo-hFSH-binding activity is significantly preserved. This effect of glycerol is time, temperature, and concentration dependent. FSH receptors extracted from calf testes with 2% Triton X-100 in the presence of 30% glycerol retained 50% of their initial binding activity after 48 h at 4 C and 60% of their initial binding activity after 30 days at -20C. **Association constants** (K(a)) measured in the presence and absence of glycerol were similar (.apprx.109 M-1). There was a **4-fold increase** in recoverable receptor activity in the presence of glycerol, although glycerol itself did not solubilize the receptor. The loss of [125I]iodo-hFSH-binding activity by the receptor with time at 4 C was characterized by a decrease in K(a), with no change in receptor concentration, indicating that this was a consequence of progressive structural or conformational alteration in the receptor rather than receptor loss. A 10-fold dilution of the glycerol-treated, detergent-solubilized receptor with 30% glycerol resulted in a more rapid loss of [125I]iodo-hFSH-binding activity than occurred with the concentrated receptor. However, even with concentrated receptor, [125I]iodo-hFSH-binding activity was lost more rapidly at 4 C than at -20 C, indicating that a temperature-related denaturation effect was also operative. Our data are consistent with a model of the FSH receptor as possessing a quaternary structure wherein the loss of [125I]iodo-hFSH-binding activity is a combination of these phenomena.

L20 ANSWER 13 OF 14 CAPLUS

1980:69912 Document No. 92:69912 Regulation of uterine angiotensin II receptors by estrogen and progesterone. Schirar, Alain; Capponi, Alessandro; Catt, Kevin J. (Natl. Inst. Child Health Hum. Dev., NIH, Bethesda, MD, 20205, USA). Endocrinology, 106(1), 5-12 (English) 1980.
CODEN: ENDOAO. ISSN: 0013-7227.

AB The characteristics of uterine receptors for angiotensin II [11128-99-7] and their regulation during the estrous cycle and after gonadal steroid treatment were investigated in the rat and rabbit. Binding of

125I-labeled iodoangiotensin II to rat uterine particles was temperature-dependent and saturable, and Scatchard anal. showed a single population of binding sites with an **association constant** of .apprx.109M-1. During the ovarian cycle, the concentration of angiotensin II receptors was .apprx.4-fold higher at proestrus than at diestrus II. Similar changes were observed when estrous cycles were induced in immature rats by a single injection of pregnant mare serum gonadotropin [9002-70-4]. Ovariectomy was followed by a progressive decrease in uterine angiotensin II receptors, reaching 50% of the control values 8 days after castration. Conversely, injection of 17β-estradiol [50-28-2] caused a dose-dependent increase in angiotensin II receptors measured 24 h after steroid treatment. Similarly, short-term infusion of 17β-estradiol caused a progressive increase in angiotensin II receptors, which reached a maximum of 4-6-fold above control values at 36 h. However, after long-term infusion of estradiol for 5-8 days, angiotensin II receptors returned to or below the control values. Progesterone [57-83-0] infusion for 7 days decreased uterine angiotensin II receptors by 93%. When immature rabbits were primed with estrogen, a **4-fold increase** in the concentration of uterine angiotensin II receptors was measured. In progesterone-primed uteri, the angiotensin II receptor concentration was no longer augmented by estrogen. No effects of steroids on the affinity of the receptors for angiotensin II were detected, all changes in binding being due to alterations in the tissue content of receptor sites. Changes in the concentration of angiotensin II receptors during the estrous cycle and after steroid treatment indicate that the receptors are regulated by estrogen in a manner similar to the control of receptors for other oxytocic hormones.

L20 ANSWER 14 OF 14 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

1979:273159 Document No.: PREV197968075663; BA68:75663. EVIDENCE FOR INTERNALIZATION OF THE RECOGNITION SITE OF BETA ADRENERGIC RECEPTORS DURING RECEPTOR SUB SENSITIVITY INDUCED BY LEVO ISOPROTERENOL. CHUANG D-M [Reprint author]; COSTA E. LAB PRECLIN PHARMACOL, NATL INST MENT HEALTH, ST ELIZABETH HOSP, WASHINGTON, DC 20032, USA. Proceedings of the National Academy of Sciences of the United States of America, (1979) Vol. 76, No. 6, pp. 3024-3028.
CODEN: PNASA6. ISSN: 0027-8424. Language: ENGLISH.

AB In the supernatant (30,000 + g) of frog erythrocyte homogenates, protein that could bind [3H]dihydroalprenolol ([3H]DHA) with high affinity was detected by gel filtration. This binding was greatly enhanced when the erythrocytes were preincubated with (-)-isoproterenol. After various periods of incubation with (-)-isoproterenol, the extent of the increase in the density of [3H]DHA binding sites in the cytosol was paralleled by a proportional decrease in the number of [3H]DHA binding sites in the corresponding pellet; both events peaked after 2-3 h of incubation with (-)-isoproterenol. The Ka [**association constant**] of the (-)-isoproterenol-induced increase in [3H]DHA binding in cytosol and the decrease in this binding in the membrane ranged between 60-90 nM. The changes in the cytosol and particulate [3H]DHA binding sites were independent of RNA and protein synthesis. The increase in cytosol binding elicited by (-)-isoproterenol was blocked by exposure of the cells to (-)-alprenolol which failed to change the cytosol binding of [3H]DHA. Scatchard analysis revealed that the enhanced [3H]DHA binding to cytosol material was due to a **4-fold increase** in the Bmax [binding maximum] with little or no change in Kd (≈ 9 nM). Binding displacement data show that the soluble [3H]DHA binding sites resemble the surface membrane recognition sites. The ability of various β-adrenergic agents to increase [3H]DHA binding to cytosol after they were incubated with frog erythrocytes paralleled their affinity for membrane-bound β receptors. Apparently the β-adrenergic receptor desensitization caused by prolonged exposure to (-)-isoproterenol is due to an internalization of the recognition site of β-adrenergic receptors.

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=> s l1 and butyrylcholinesterase
L21          2 L1 AND BUTYRYLCHOLINESTERASE

=> dup remove l21
PROCESSING COMPLETED FOR L21
L22          1 DUP REMOVE L21 (1 DUPLICATE REMOVED)

=> d l22 cbib abs

L22  ANSWER 1 OF 1  SCISEARCH  COPYRIGHT (c) 2006 The Thomson Corporation  on
     STN                                                        DUPLICATE 1
2001:556650   The Genuine Article (R) Number: 449XP. Acoustic and optical
     transduction of BuChE binding to procainamide modified surfaces.  Marx S;
     Kaushansky N; Gratziany N; Barness I; Liron Z (Reprint). Israel Inst Biol
     Res, Dept Phys Chem, POB 19, IL-70450 Ness Ziona, Israel (Reprint); Israel
     Inst Biol Res, Dept Phys Chem, IL-70450 Ness Ziona, Israel. BIOSENSORS &
     BIOELECTRONICS (JUN 2001) Vol. 16, No. 4-5, pp. 239-244. ISSN: 0956-5663.
     Publisher: ELSEVIER ADVANCED TECHNOLOGY, OXFORD FULFILLMENT CENTRE THE
     BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND.
     Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB        A novel polymer, poly(procainamide), PPA, containing numerous binding
     sites for cholinesterases was synthesized as a recognition layer for
     butyryl cholinesterase (BuChE) interaction with the ligand procainamide,
     utilizing TSM and SPR sensors.  The polymer was synthesized by the
     reaction of methacryloyl chloride and procainamide followed by radical
     polymerization.  Sensor surfaces (Au or SiO2) were spin-coated by the
     polymer solution to form thin layers.  Binding of BuChE was found to be
     sensitive to the drying procedure of the polymer layer.  The binding of
     BuChE to the polymer coated sensors was monitored on-line by following the
     response of thickness shear mode (TSM) and surface plasmon resonance (SPR)
     sensors.  Binding of BuChE to PPA-coated TSM sensors were shown to follow
     a Langmuir isotherm giving association constant 3.4 x
     10(6) M-1 (C) 2001 Elsevier Science B.V.  All rights reserved.


=> s peptide libraries
L23        5003 PEPTIDE LIBRARIES

=> s l23 and "Kon"
L24          1 L23 AND "KON"

=> d l24 cbib abs

L24  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2006 ACS on STN
1997:332328   Document No. 127:134458  Mimotopes of polyreactive anti-DNA
     antibodies identified using phage-display peptide
     libraries.  Sibille, Pierre; Ternynck, Therese; Nato, Faridabano;
     Buttin, Gerard; Strosberg, Donny; Avrameas, Alexandre (Institut Cochin
     Genetique Moleculaire, Paris, Fr.).  European Journal of Immunology,
     27(5), 1221-1228 (English) 1997.  CODEN: EJIMAF.  ISSN: 0014-2980.
     Publisher: VCH.
AB   Three monoclonal IgG2a anti-DNA polyreactive autoantibodies, derived from
     lupus-prone mice (NZB + NZW)F1, were studied by surface plasmon
     resonance (BIAcore) anal. using 3 different synthetic double-stranded (ds)
     oligonucleotides of 25, 30, and 25 base pairs (bp).  These monoclonal
     antibodies (mAb) exhibited dissociation rate consts. (koff) ranging from
     0.0001 (mAb F14.6 and F4.1) to 0.01/s (mAb J20.8) and kon
     ranging from 2+105 to 2+106 /M/s.  The screening of a
     constrained random peptide library displayed on M13 bacteriophages on
     these mAb allowed the determination of the specific consensus motifs
(mimotopes)
     for mAb F14.6 and J20.8, but not for mAb F4.1.  No cross-reaction was
     observed between F14.6- and J20.8-specific peptides (and vice versa).
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Binding of all phages selected on F14.6 was inhibited with 700 ng/mL soluble DNA. The binding of a group of peptides selected on J20.8 was inhibited by 400 ng/mL soluble DNA, of a 2nd group by 2500 ng/mL, while binding of a 3rd group was not inhibited. The determined consensus sequences do not match with known sequences. Peptides specific for F14.6 share neg. charges and aromatic rings that may mimic a DNA backbone, while peptides selected with J20.8 do not bear any neg. charge, implying a different kind of mol. recognition, for example hydrogen or salt bonds. The peptides selected on J20.8 also bind serum antibodies from human patients with systemic lupus erythematosus. BALB/c mice immunized with some of the selected phages exhibit high serum titers of IgG3 anti-dsDNA antibodies, further supporting the hypothesis that peptide epitopes may mimic an oligonucleotide structure.

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=> s l23 and association rate
L25           0 L23 AND ASSOCIATION RATE

=> s l23 and screening
L26        1663 L23 AND SCREENING

=> s l26 and binding affinity
L27          67 L26 AND BINDING AFFINITY

=> s l27 and higher association rate
L28           0 L27 AND HIGHER ASSOCIATION RATE

=> s l27 and association rate
L29           0 L27 AND ASSOCIATION RATE

=> s l26 and association rate
L30           0 L26 AND ASSOCIATION RATE

=> s l23 and association rate
L31           0 L23 AND ASSOCIATION RATE

=> s association rate
L32        9828 ASSOCIATION RATE

=> s l32 and improvement
L33          56 L32 AND IMPROVEMENT

=> dup remove l33
PROCESSING COMPLETED FOR L33
L34           16 DUP REMOVE L33 (40 DUPLICATES REMOVED)

=> d l34 1-16 cbib abs

L34  ANSWER 1 OF 16  CAPLUS  COPYRIGHT 2006 ACS on STN
2005:1089103  Ultra-potent antibodies against respiratory syncytial virus:.
     Wu, H.; Pfarr, D. S.; Tang, Y.; An, L-L.; Pate, N. K.; Watkins, J. D.;
     Huse, W. D.; Kiener, P. A.; Young, J. F. (USA).  Assay and Drug
     Development Technologies, 3(4), 450-452 (English) 2005.  CODEN: ADDTAR.
     ISSN: 1540-658X.  Publisher: Mary Ann Liebert, Inc..
AB   We describe there the selection of ultra-potent anti-respiratory syncytial
     virus (RSV) antibodies for preventing RSV infection.  A large number of
     antibody variants derived from Synagis (palivizumab), an anti-RSV
     monoclonal antibody that targets RSV F protein, were generated by a
     directed evolution approach that allowed convenient manipulation of the
     binding kinetics.  Palivizumab variants with about 100-fold slower
     dissociation rates or with fivefold faster association rates
     were identified and tested for their ability to neutralize virus in a
     microneutralization assay.  Our data reveal a major differential effect of
     the association and dissociation rates on the RSV neutralization, particularly
for
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intact antibodies wherein the **association rate** plays the predominant role. Furthermore, we found that antibody binding valence also plays a critical role in mediating the viral neutralization through a mechanism that is likely unrelated to antibody size or binding avidity. We applied an iterative mutagenesis approach, and thereafter were able to identify palivizumab Fab variants with up to 1,500-fold **improvement** and palivizumab IgG variants with up to 44-fold **improvement** in the ability to neutralize RSV. These anti-RSV immunoprophylaxis. In addition, our findings provide insights into engineering potent antibody therapeutics for other disease targets.

L34 ANSWER 2 OF 16 MEDLINE on STN DUPLICATE 1
2005293960. PubMed ID: 15907931. Ultra-potent antibodies against respiratory syncytial virus: effects of binding kinetics and binding valence on viral neutralization. Wu Herren; Pfarr David S; Tang Ying; An Ling-Ling; Patel Nita K; Watkins Jeffry D; Huse William D; Kiener Peter A; Young James F. (MedImmune, Inc., One MedImmune Way, Gaithersburg, MD 20878, USA.. wuh@medimmune.com) . Journal of molecular biology, (2005 Jul 1) 350 (1) 126-44. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB We describe here the selection of ultra-potent anti-respiratory syncytial virus (RSV) antibodies for preventing RSV infection. A large number of antibody variants derived from Synagis (palivizumab), an anti-RSV monoclonal antibody that targets RSV F protein, were generated by a directed evolution approach that allowed convenient manipulation of the binding kinetics. Palivizumab variants with about 100-fold slower dissociation rates or with fivefold faster **association rates** were identified and tested for their ability to neutralize virus in a microneutralization assay. Our data reveal a major differential effect of the association and dissociation rates on the RSV neutralization, particularly for intact antibodies wherein the **association rate** plays the predominant role. Furthermore, we found that antibody binding valence also plays a critical role in mediating the viral neutralization through a mechanism that is likely unrelated to antibody size or binding avidity. We applied an iterative mutagenesis approach, and thereafter were able to identify palivizumab Fab variants with up to 1500-fold **improvement** and palivizumab IgG variants with up to 44-fold **improvement** in the ability to neutralize RSV. These anti-RSV antibodies likely will offer great clinical potential for RSV immunoprophylaxis. In addition, our findings provide insights into engineering potent antibody therapeutics for other disease targets.

L34 ANSWER 3 OF 16 MEDLINE on STN DUPLICATE 2
2004172831. PubMed ID: 14754891. "Network leaning" as a mechanism of insurmountable antagonism of the angiotensin II type 1 receptor by non-peptide antagonists. Takezako Takanobu; Gogonea Camelia; Saad Yasser; Noda Keita; Karnik Sadashiva S. (Department of Molecular Cardiology, Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA.) Journal of biological chemistry, (2004 Apr 9) 279 (15) 15248-57. Electronic Publication: 2004-01-30. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A mechanistic understanding of the insurmountable antagonism of the angiotensin II type 1 (AT(1)) receptor could be fundamental in the quest for discovery and **improvement** of drugs. Candesartan and EXP3174 are competitive, reversible insurmountable antagonists of the AT(1) receptor. They contain di-acidic substitutions, whereas the surmountable antagonist, losartan, contains only one acidic group. We tested the hypothesis that these two classes of ligands interact with the AT(1) receptor through similar but not identical bonds and that the differences in the acid-base group contacts are critical for insurmountable antagonism. By pharmacological characterization of site-directed AT(1) receptor mutants expressed in COS1 cells we show that specific interactions with Gln(257) in transmembrane 6 distinguishes insurmountable antagonists and that abolishing these interactions transforms

insurmountable to surmountable antagonism. In the Q257A mutant, the dissociation rate of [(3)H]candesartan is 2.8-fold more than the rate observed with wild type, and the **association rate** was reduced 4-fold lower than the wild type. The pattern of antagonism of angiotensin II concentration-response in the Q257A mutant pretreated with EXP3174 and candesartan is surmountable. We propose that leaning ability of insurmountable antagonists on Gln(257) in the wild-type receptor is the basis of an antagonist-mediated conformational transition, which is responsible for both slow dissociation and inhibition of maximal IP response.

L34 ANSWER 4 OF 16 MEDLINE on STN DUPLICATE 3
2004144036. PubMed ID: 15037082. Fucose depletion from human IgG1 oligosaccharide enhances binding enthalpy and **association rate** between IgG1 and FcgammaRIIIa. Okazaki Akira; Shoji-Hosaka Emi; Nakamura Kazuyasu; Wakitani Masako; Uchida Kazuhisa; Kakita Shingo; Tsumoto Kouhei; Kumagai Izumi; Shitara Kenya. (Tokyo Research Laboratories, Kyowa Hakko Kogyo Co Ltd, 3-6-6 Asahi-machi, Machida-shi, Tokyo 194-8533, Japan.) Journal of molecular biology, (2004 Mar 5) 336 (5) 1239-49. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB Depletion of fucose from human IgG1 oligosaccharide improves its affinity for Fcgamma receptor IIIa (FcgammaRIIIa). This is the first case where a glycoform modification is shown to improve glycoprotein affinity for the receptors without carbohydrate-binding capacity, suggesting a novel glyco-engineering strategy to improve ligand-receptor binding. To address the mechanisms of affinity **improvement** by the fucose depletion, we used isothermal titration calorimetry (ITC) and biosensor analysis with surface plasmon resonance. ITC demonstrated that IgG1-FcgammaRIIIa binding was driven by favorable binding enthalpy (DeltaH) but opposed by unfavorable binding entropy change (DeltaS). Fucose depletion from IgG1 enhanced the favorable DeltaH, leading to the increase in the binding constant of IgG1 for the receptor by a factor of 20-30. The increase in the affinity was mainly attributed to an enhanced **association rate.** A triple amino acid substitution in IgG1, S298A/E333A/K334A, is also known to improve IgG1 affinity for FcgammaRIIIa. ITC demonstrated that the amino acid substitution attenuated the unfavorable DeltaS resulting in a three- to fourfold increase in the binding constant. The affinity enhancement by the amino acid substitution was due to a reduced dissociation rate. These results indicate that the mechanism of affinity **improvement** by the fucose depletion is quite distinct from that by the amino acid substitution. Defucosylated IgG1 exhibited higher antibody-dependent cellular cytotoxicity (ADCC) than S298A/E333A/K334A-IgG1, showing a correlation between IgG1 affinity for FcgammaRIIIa and ADCC. We also examined the effect of FcgammaRIIIa polymorphism (Val158/Phe158) on IgG1-FcgammaRIIIa binding. The Phe to Val substitution increased FcgammaRIIIa affinity for IgG1 in an enthalpy-driven manner with the reduced dissociation rate. These results together highlight the distinctive functional **improvement** of affinity by IgG1 defucosylation and suggest that engineering of non-interfacial monosaccharides can improve glycoprotein affinity for receptors via an enthalpy-driven and **association rate**-assisted mechanism.

L34 ANSWER 5 OF 16 MEDLINE on STN DUPLICATE 4
2004497170. PubMed ID: 15465055. Substantial energetic **improvement** with minimal structural perturbation in a high affinity mutant antibody. Midelfort K S; Hernandez H H; Lippow S M; Tidor B; Drennan C L; Wittrup K D. (Biological Engineering Division, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.) Journal of molecular biology, (2004 Oct 22) 343 (3) 685-701. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB Here, we compare an antibody with the highest known engineered affinity (K(d)=270 fM) to its high affinity wild-type (K(d)=700 pM) through

thermodynamic, kinetic, structural, and theoretical analyses. The 4M5.3 anti-fluorescein single chain antibody fragment (scFv) contains 14 mutations from the wild-type 4-4-20 scFv and has a 1800-fold increase in fluorescein-binding affinity. The dissociation rate is approximately 16,000 times slower in the mutant; however, this substantial **improvement** is offset somewhat by the **association rate**, which is ninefold slower in the mutant. Enthalpic contributions to binding were found by calorimetry to predominate in the differential binding free energy. The crystal structure of the 4M5.3 mutant complexed with antigen was solved to 1.5A resolution and compared with a previously solved structure of an antigen-bound 4-4-20 Fab fragment. Strikingly, the structural comparison shows little difference between the two scFv molecules (backbone RMSD of 0.6A), despite the large difference in affinity. Shape complementarity exhibits a small **improvement** between the variable light chain and variable heavy chain domains within the antibody, but no significant **improvement** in shape complementarity of the antibody with the antigen is observed in the mutant over the wild-type. Theoretical modeling calculations show electrostatic contributions to binding account for -1.2 kcal/mol to -3.5 kcal/mol of the binding free energy change, of which -1.1 kcal/mol is directly associated with the mutated residue side-chains. The electrostatic analysis reveals several mechanistic explanations for a portion of the **improvement.** Collectively, these data provide an example where very high binding affinity is achieved through the cumulative effect of many small structural alterations.

L34 ANSWER 6 OF 16 MEDLINE on STN DUPLICATE 5

2002249122. PubMed ID: 11988096. Engineering N-terminal domain of tissue inhibitor of metalloproteinase (TIMP)-3 to be a better inhibitor against tumour necrosis factor-alpha-converting enzyme. Lee Meng-Huee; Verma Vandana; Maskos Klaus; Nath Deepa; Knauper Vera; Dodds Philippa; Amour Augustin; Murphy Gillian. (School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.. meng.lee@uea.ac.uk) . Biochemical journal, (2002 May 15) 364 (Pt 1) 227-34. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: England: United Kingdom. Language: English.

AB We previously reported that full-length tissue inhibitor of metalloproteinase-3 (TIMP-3) and its N-terminal domain form (N-TIMP-3) displayed equal binding affinity for tissue necrosis factor-alpha (TNF-alpha)-converting enzyme (TACE). Based on the computer graphic of TACE docked with a TIMP-3 model, we created a number of N-TIMP-3 mutants that showed significant **improvement** in TACE inhibition. Our strategy was to select those N-TIMP-3 residues that were believed to be in actual contact with the active-site pockets of TACE and mutate them to amino acids of a better-fitting nature. The activities of these mutants were examined by measuring their binding affinities (K(app)(i)) and **association rates** (k(on)) against TACE. Nearly all mutants at position Thr-2 exhibited slightly impaired affinity as well as **association rate** constants. On the other hand, some Ser-4 mutants displayed a remarkable increase in their binding tightness with TACE. In fact, the binding affinities of several mutants were less than 60 pM, beyond the sensitivity limits of fluorimetric assays. Further studies on cell-based processing of pro-TNF-alpha demonstrated that wild-type N-TIMP-3 and one of its tight-binding mutants, Ser-4Met, were capable of inhibiting the proteolytic shedding of TNF-alpha. Furthermore, the Ser-4Met mutant was also significantly more active ($P<0.05$) than the wild-type N-TIMP-3 in its cellular inhibition. Comparison of N-TIMP-3 and full-length TIMP-3 revealed that, despite their identical TACE-interaction kinetics, the latter was nearly 10 times more efficient in the inhibition of TNF-alpha shedding, with concomitant implications for the importance of the TIMP-3 C-terminal domain in vivo.

L34 ANSWER 7 OF 16 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2001:493683 Document No.: PREV200100493683. Electrostatic control of protein-protein docking and electron transfer. Liang, Zhao-Xun [Reprint author]; Nocek, Judith M. [Reprint author]; Mauk, A. Grant; Hoffman, Brian

M. [Reprint author]. Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL, 60208, USA. zxliang@chem.nwu.edu. Abstracts of Papers American Chemical Society, (2001) Vol. 222, No. 1-2, pp. BIOL53. print.
Meeting Info.: 222nd National Meeting of the American Chemical Society. Chicago, Illinois, USA. August 26-30, 2001. American Chemical Society. CODEN: ACSRAL. ISSN: 0065-7727. Language: English.

AB Most of the cell functions are regulated by protein-protein interactions with delicately balanced binding affinity and specificity. Due to the weak affinity and transient nature of the protein complexes involved in many of those protein-protein interactions, both structural and dynamic information are still elusive. In this study, with a model system consists of zinc substituted myoglobin (ZnMb) and cytochrome b5, we demonstrate that the modification of the surface charge of ZnMb by means of chemical modification and site-directed mutagenesis causes profound changes in protein-protein docking specificity and electron transfer (ET) rate. With the help of electrostatic calculations and Brownian dynamic simulations, we show that the **improvement** of the docking specificity results from the optimization of the electrostatic interactions at the docking interface. This work suggests that the optimal local electrostatic interactions are critical for protein-protein docking to achieve high specificity and modest binding affinity required for fast association/dissociation rate. Meanwhile, this study also suggests that photoinduced electron transfer may be used as a practical tool for studying transient protein-protein interactions.

L34 ANSWER 8 OF 16 CAPLUS COPYRIGHT 2006 ACS on STN

2000:163472 Document No. 133:116938 Improved response of a fluorescence-based metal ion biosensor using engineered carbonic anhydrase variants. Thompson, Richard B.; Zeng, Hui-Hui; Loetz, Michele; McCall, Keith; Fierke, Carol A. (Dep. Biochem. Mol. Biol., Univ. of Maryland, Sch. Med., Baltimore, MD, USA). Proceedings of SPIE-The International Society for Optical Engineering, 3858(Advanced Materials and Optical Systems for Chemical and Biological Detection), 161-166 (English) 1999. CODEN: PSISDG. ISSN: 0277-786X. Publisher: SPIE-The International Society for Optical Engineering.

AB The response time of biosensors which reversibly bind an analyte such as a metal ion is necessarily limited by the kinetics with which the biosensor transducer binds the analyte. In the case of the carbonic anhydrase-based biosensor we have developed the binding kinetics are rather slow, with the wild type human enzyme exhibiting an **association rate** constant ten thousand-fold slower than diffusion-controlled. By designed and combinatorial means the transducer may be mutagenized to achieve nearly diffusion-controlled **association rate** consts., with commensurate **improvement** in response. In addition, a variant of apocarbonic anhydrase was immobilized on quartz, and is shown to response rapidly to changes in free copper ion in the picomolar range.

L34 ANSWER 9 OF 16 MEDLINE on STN DUPLICATE 6

97185711. PubMed ID: 9033392. Contribution of lysine 60f to S1' specificity of thrombin. Rezaie A R; Olson S T. (Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City 73104, USA.) Biochemistry, (1997 Feb 4) 36 (5) 1026-33. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Lys60f has been proposed to limit the S1' substrate binding site specificity of thrombin to small polar P1' residues by occluding the S1' binding pocket, based on the X-ray crystal structure of thrombin. To test this proposal, we prepared a Lys-->Ala (K60fA) mutant of recombinant thrombin and determined whether this mutation enhanced the reactivity of thrombin with a variant inhibitor [antithrombin (AT)-Denver] and a substrate (protein C) containing poorly recognized P1' Leu residues. AT-Denver in the presence of heparin inhibited K60fA thrombin with a second-order **association rate** constant [k = 4.2 +/- 0.1) x 10(5) M-1 s-1] that was 3.2-fold faster than thrombin [k = (1.3 +/- 0.1) x 10(5) M-1 s-1]. Wildtype AT (P1' Ser) under the same conditions

inhibited K60fA thrombin with a 2.5-fold slower rate constant [k = (1.1 +/- 0.1) x 10(7) M-1 s-1] than thrombin [k = (2.8 +/- 0.1) x 10(7) M-1 s-1]. These results indicate an overall 8.3-fold **improvement** in the recognition of the P1' Leu of AT-Denver by K60fA thrombin over that of wild-type thrombin; i.e., the K60fA mutation partly overcomes the defect in thrombin inhibition produced by the P1' mutation in AT-Denver. Resolution of the two-step reactions of AT and AT-Denver with wild-type and mutant thrombins revealed that the enhanced recognition of P1' Leu in AT-Denver by K60fA thrombin occurs primarily in the second reaction step in which a noncovalent AT-thrombin encounter complex is converted to a stable, covalent complex. Thrombin K60fA activated Gla-domainless protein C (GDPC) approximately 2- and approximately 4-fold faster than thrombin in the presence and absence of thrombomodulin (TM), respectively, consistent with an improved interaction of the Leu P1' residue with the mutant S1' pocket. In contrast, the mutant thrombin clotted fibrinogen (P1' Gly) approximately 3-fold slower than thrombin. Kinetic analysis revealed that the **improvement** in the catalytic rate of activation of GDPC by K60fA thrombin in the presence of TM was localized in the second reaction step, as reflected by an approximately 2-fold increase in kcat. Direct binding studies showed that the K60fA mutation minimally affected the affinity of thrombin for Na+, indicating that the changes in S1' site-specificity of K60fA thrombin did not result from altering the allosteric transition induced by Na+. We conclude that Lys60f limits the P1' substrate and inhibitor specificity of thrombin by influencing the size and polarity of the S1' site which thereby affects the stability of the transition state for cleavage of the scissile bond in the second reaction step.

L34 ANSWER 10 OF 16 MEDLINE on STN DUPLICATE 7

97110321. PubMed ID: 8952482. Efficient **improvement** of hammerhead ribozyme mediated cleavage of long substrates by oligonucleotide facilitators. Jankowsky E; Schwenzer B. (Institut fur Biochemie, Technische Universitat Dresden, Germany.). Biochemistry, (1996 Dec 3) 35 (48) 15313-21. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Hammerhead ribozymes were found to be not very efficient in cleaving long RNA substrates in trans. Oligonucleotide facilitators, capable of affecting hammerhead ribozymes by interacting with the substrate at the termini of the ribozyme, may improve this reaction. We determined in vitro the effects of 18 DNA and RNA oligonucleotide facilitators on three substrates containing 39, 452, and 942 nucleotides, respectively, by estimating the facilitator influences on association between ribozyme and substrate and on the cleavage step. The effects increase with the length of the substrates. With the 39mer substrate a maximal 4-fold enhancement of the ribozyme activity could be detected, the reaction with the 942mer substrate was accelerated up to 115-fold by facilitator addition. In long, structured substrates the facilitators have the potential to preform the substrate for the ribozyme attack. Due to this preforming effect, the rate of ribozyme-substrate association was increased as well as the rate of the cleavage step. 3'-End facilitators accelerate both of these rates, largely independent on the facilitator length. The rate of the cleavage step is raised as a result of a favorable activation energy gain by these facilitators. With all substrates, the 5'-end facilitators increase the **association rate** between ribozyme and substrate in dependence on their length. With the 39mer substrate the 5'-end facilitators decrease the rate of the cleavage step. With the long substrates 5'-end facilitators partially increase the rate of the cleavage step due to their preforming potential with these substrates. In some examples, combinations of several 5'-end and 3'-end facilitators provide an additional **improvement** over single facilitators in both the association between ribozyme and substrate and the cleavage step. Results suggest that even short facilitators may be efficient effectors enhancing hammerhead ribozyme mediated cleavage of long substrates.

L34 ANSWER 11 OF 16 MEDLINE on STN DUPLICATE 8

95263530. PubMed ID: 7744836. Development of a novel recombinant serpin with potential antithrombotic properties. Hopkins P C; Crowther D C; Carrell R W; Stone S R. (Department of Haematology, University of Cambridge, United Kingdom.) Journal of biological chemistry, (1995 May 19) 270 (20) 11866-71. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Recombinant alpha 1-antitrypsin with a P1 arginine residue (Arg-alpha 1-antitrypsin) is a rapid inhibitor of both thrombin and activated protein C (APC). A series of mutants were made in an attempt to increase the specificity of this serpin for thrombin over APC. Initially, P2 and P'1 residues of Arg-alpha 1-antitrypsin were replaced in single and double mutations by the corresponding residues in antithrombin and C1 inhibitor which are very poor inhibitors of APC. No **improvement** in selectivity was achieved by these mutations. In fact, all P2/P'1 substitutions led to a decrease in selectivity for thrombin over APC. For example, replacement of the P2 proline of Arg-alpha 1-antitrypsin by glycine decreased the **association rate** constant (kass) with thrombin by 37-fold while the kass value with APC was reduced by only 16-fold. Cooperative effects were observed with the double P2 and P'1 substitutions; the mutational effects were not additive. The decrease in the kass for thrombin caused by the mutation of the P2 proline to alanine or glycine was 3-fold greater when threonine was present in the P'1 position instead of the normal serine. In contrast to the disappointing results with the P2/P'1 mutations, replacement of the P7 to P'3 residues of alpha 1-antitrypsin by those of antithrombin led to a dramatic increase in selectivity. Although this substitution only affected the kass value with thrombin by 10-fold, a 12,500-fold decrease in this value with APC was observed. Substitution of proline for the P2 glycine of this chimeric serpin increased the kass values with thrombin and APC by 7- and 90-fold, respectively. The effect of the P2 substitution was again found to depend on the sequence surrounding the residue; the change in the kass for APC caused by the P2 Pro-->Gly replacement was 6-fold larger in the chimeric serpin. Evaluation of the kass values of the chimeric serpin with a P2 proline in light of the likely rates of inhibition of thrombin and APC during antithrombotic therapy with heparin suggested that this serpin may have kinetic parameters suitable for an antithrombotic agent.

L34 ANSWER 12 OF 16 MEDLINE on STN DUPLICATE 9

95286993. PubMed ID: 7769242. Characterization of the interaction of alkaline phosphatase with an activity inhibiting monoclonal antibody by progress curve analysis. Cumme G A; Walter U; Bublitz R; Hoppe H; Rhode H; Horn A. (Institute of Biochemistry, Friedrich Schiller University, Jena, Germany.) Journal of immunological methods, (1995 May 11) 182 (1) 29-39. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Using the enzyme activity inhibiting monoclonal antibody IB 10B8 against alkaline phosphatase of calf intestine (AP), the interaction of a macromolecular antigen with the antibody was studied with different reaction conditions and with different conformations of the antigen, i.e. using (i) different pH values, (ii) different temperatures, (iii) different substrate saturation of the enzyme, (iv) different glycosylphosphatidyl-AP (GPI-AP) aggregates, and (v) membrane-bound species. In the case of antibody excess and negligible substrate consumption enzymic product formation proceeds according to [P] = a + b x t - c x exp(-d x t). By direct progress curve fitting and secondary data evaluation using nonlinear regression, omitting numerical derivation and graphic techniques, kinetic constants of the immune reaction have been estimated. The method does not require any artificial labelling nor any separation of bound and free entities. (i) Upon increasing pH from 9.8 to 11.0, the dissociation constant of the enzyme-antibody complex is increased strongly, mainly due to the decreasing **association rate** constant. (ii) A temperature increase from 25 degrees C to 37 degrees C produces a marked increase of both the association and dissociation rate constant. (iii) To differentiate between the interaction of the antibody with the free (E) and substrate-bound (ES) enzyme,

experiments were done at different substrate concentrations. The results were fitted to a model allowing determination of association and dissociation rate constants of the free and substrate-bound enzyme. The inverse variation of association and dissociation rate constants caused by substrate binding produces a marked increase of the dissociation constant of the antibody-enzyme complex. The antibody-bound enzyme shows a nearly three-fold higher Km value and a six-fold lower catalytic constant as compared to the free enzyme. (iv) Investigations of the interaction of the antibody with anchorless AP, different hydrophobic aggregates of purified GPI-AP (fractions II-V). (v) Membrane-bound GPI-AP show that the epitopes of all species are fully accessible to the antibody and not cryptic. Surprisingly the insertion of the GPI-moiety into the membrane and the aggregation of the different GPI-AP fractions II-V seem to improve antibody binding. Such **improvement** of binding was not found in control experiments with Fab, indicating only for the bivalent antibody a stronger interaction with the multivalent antigen than with the monovalent antigen.

L34 ANSWER 13 OF 16 MEDLINE on STN DUPLICATE 10

92028940. PubMed ID: 1930217. Calcium binding to fluorescent calcium indicators: calcium green, calcium orange and calcium crimson. Eberhard M; Erne P. (Department of Research, Kantonsspital, Basel, Switzerland.) Biochemical and biophysical research communications, (1991 Oct 15) 180 (1) 209-15. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB The recently introduced fluorescent calcium sensitive indicators calcium green, calcium orange and calcium crimson suggest important **improvements** and advantages to detect small calcium transients at low indicator concentrations. Thermodynamic dissociation constants and dissociation rate constants of calcium green, calcium orange and calcium crimson were measured by use of fluorescence titration and stopped flow fluorescence, respectively. Calcium binding to the indicators conforms to a 1:1 calcium:indicator complex although at high concentrations of calcium the fluorescence properties deviate somewhat from the behaviour predicted by the simple model. Dissociation of the calcium-indicator complex was found to be monoexponential under all conditions examined. The affinity for calcium of the three indicators generally increases with raising temperatures (Kd at 11.5 degrees C and 39.7 degrees C (nM): 261, 180 for calcium green; 527, 323 for calcium orange; 261, 204 for calcium crimson) and pH (Kd at pH 6.42 and 7.40 (nM): 314, 226 for calcium green; 562, 457 for calcium orange; 571, 269 for calcium crimson). The changes of the thermodynamic dissociation constant are mainly caused by changes of the **association rate** constant. The temperature dependence of calcium binding to the indicators revealed that this process is entropically favoured at ambient temperature.

L34 ANSWER 14 OF 16 MEDLINE on STN DUPLICATE 11

90042304. PubMed ID: 2811365. **Improvement** in glucocorticoid receptor binding affinity concomitant to shift from antagonist to agonist activity in a series of 17 beta-carboxamide derivatives of dexamethasone. Lefebvre P; Formstecher P; Rousseau G G; Lustenberger P; Dautrevaux M. (Laboratoire de Biochimie Structurale, Faculte de Medecine, Lille, France.) Journal of steroid biochemistry, (1989 Oct) 33 (4A) 557-63. Journal code: 0260125. ISSN: 0022-4731. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Modification of the 17 beta-side chain of the synthetic glucocorticoid agonist dexamethasone by periodic oxidation and subsequent coupling to various primary amines yield secondary 17 beta-carboxamide derivatives displaying antiglucocorticoid activity in vitro, but not in vivo. To obtain more potent antiglucocorticoids, new secondary and tertiary 17 beta-carboxamide derivatives were synthesized. Although they displayed an improved affinity for the glucocorticoid receptor in rat thymus cytosol and antiglucocorticoid activity in rat hepatoma (HTC) cells, these new compounds were again devoid of in vivo antiglucocorticoid activity in the rat. Moreover, the increase in receptor binding affinity was correlated

for most compounds with the appearance of a partial agonist activity in HTC cells. The tertiary 17 beta-carboxamide derivative DX diMe displayed the highest affinity but was also a partial agonist in vivo. Kinetic studies with several tritiated 17 beta-carboxamide derivatives showed that they had **association rate** constants similar to that of dexamethasone, but different dissociation rate constants. The rapid dissociation of the compounds displaying antiglucocorticoid activity contrasted with the slow dissociation of DX diMe. Therefore, antiglucocorticoid activity in the 17 beta-carboxamide series is probably related to the formation of rapidly dissociating glucocorticoid receptor-ligand complexes that are unable to undergo the transformation step.

L34 ANSWER 15 OF 16 CAPLUS COPYRIGHT 2006 ACS on STN
1988:158573 Document No. 108:158573 Search for helium hydride cation (HeH+) in NGC 7027. Moorhead, J. M.; Lowe, R. P.; Maillard, J. P.; Wehlau, W. H.; Bernath, P. F. (Univ. West. Ontario, London, ON, N6A 3K7, Can.). Astrophysical Journal, 326(2, Pt. 1), 899-904 (English) 1988. CODEN: ASJOAB. ISSN: 0004-637X.
AB The 3.3 μm spectrum was studied of the planetary nebula NGC 7027 for the R(0) line of the fundamental vibration-rotation band of the mol. ion HeH+ without detecting it. The upper limit for detection is 3.7 + 10-14 ergs cm-2 s-1, an **improvement** in excess of 100 over previous published attempts. This limit is low when compared to expectations based on anal. of mol. processes in gaseous nebula. Likely causes are incorrect radiative **association rates** or inadequate representation of the nebular size or of the d. distribution in the outer boundary of the nebular model used for the flux prediction.

L34 ANSWER 16 OF 16 CAPLUS COPYRIGHT 2006 ACS on STN
1939:56370 Document No. 33:56370 Original Reference No. 33:8091f-g Some remarks on the theory of reaction rates. Wigner, Eugene P. Journal of Chemical Physics, 7, 646-52 (Unavailable) 1939. CODEN: JCPSA6. ISSN: 0021-9606.
AB Effects connected with a more complicated nature of the energy surfaces of relatively simple reactions are discussed. Better results are obtained in calculating the rate of association of atoms when 3 addnl. attraction states are considered. The calculated and exptl. rates of association of I, Br and Cl atoms are compared. The **improvement** expected from consideration of the angular momentum of the associating pair of atoms is discussed. Discussion by others is included.

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L39 ANSWER 1 OF 4     MEDLINE on STN                    DUPLICATE 1
2000051023.    PubMed ID: 10581149.   Activation of soluble guanylate cyclase
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by carbon monoxide and nitric oxide: a mechanistic model. Sharma V S; Magde D. (Department of Medicine and Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, California, 92093-0652, USA.) Methods (San Diego, Calif.), (1999 Dec) 19 (4) 494-505. Journal code: 9426302. ISSN: 1046-2023. Pub. country: United States. Language: English.

AB Soluble guanylate cyclase (GC) from bovine lung is activated 4-fold by carbon monoxide (CO) and 400-fold by nitric oxide (NO). Spectroscopic and kinetic data for ligation of CO and NO with GC are summarized and compared with similar data for myoglobin (Mb), hemoglobin (Hb), and heme model compounds. Kinetic, thermodynamic, and structural data form a basis on which to construct a model for the manner in which the two ligands affect protein structure near the heme for heme proteins in general and for GC in particular. The most significant datum is that although **association rates** of ligands with GC are similar to those with Mb and Hb, their dissociation rates are dramatically faster. This suggests a delicate balance between five- and six-coordinate heme iron in both NO and CO complexes. Based on these and other data, a model for GC activation is proposed: The first step is formation of a six-coordinate species concomitant with tertiary and quaternary structural changes in protein structure and about a **4-fold increase** in **enzyme** activity. In the second step, applicable to NO, the bond from iron to the proximal histidine ruptures, leading to additional relaxation in the quaternary and tertiary structure and a further 100-fold **increase** in activity. This is the main event in activation, available to NO and possibly other activators or combinations of activators. It is proposed, finally, that the proximal base freed in step 2, or some other protein base suitably positioned as a result of structural changes following ligation, may provide a center for nucleophilic substitution catalyzing the reaction GTP --> cGMP. An example is provided for a similar reaction in a derivatized protoheme model compound. The reaction mechanism attempts to rationalize the relative enzymatic activities of GC, heme-deficient GC, GC-CO, and GC-NO on a common basis and makes predictions for new activators that may be discovered in the future.

L39 ANSWER 2 OF 4 MEDLINE on STN DUPLICATE 2

94327590. PubMed ID: 8051119. Sugar transport by the bacterial phosphotransferase system. Characterization of the Escherichia coli **enzyme** I monomer/dimer transition kinetics by fluorescence anisotropy. Chauvin F; Brand L; Roseman S. (McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland 21218.) Journal of biological chemistry, (1994 Aug 12) 269 (32) 20270-4. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB **Enzyme** I of the bacterial phosphoenolpyruvate: glycose phosphotransferase system (PTS) exists in a monomer/dimer (M/D) equilibrium. These two forms are functionally different, and their interconversion may be a means of regulating the PTS. The M/D equilibrium was studied by fluorescence anisotropy of a pyrene derivative (Chauvin, F., Brand, L., and Roseman, S. (1994) J. Biol. Chemical 269, 20263-20269). In this paper, the kinetics of the transition is investigated. The following apparent rate constants were found for the M/D transition of phospho-**Enzyme** I in the presence of Mg2+ and PEP at 6 degrees C: k*A = 3.4 x 10(3) M-1 s-1 and k*D = 1.04 x 10(3) s-1. The **association rate** is especially slow, 2-3 orders of magnitude slower than the average dimerization rate determined for other proteins. Furthermore, the rate of quaternary structure changes matches that of enzymatic activity changes, as well as that of tertiary structure changes (Chauvin, F., Toptygin, D., Roseman, S., and Brand, L. (1992) Biophys. Chemical 44, 163-173). Finally, the effect of two ligands is shown; PEP **increases** the relaxation rate by 3-fold at 23 degrees C, and Mg2+ addition causes a **4-fold increase** in the relaxation rate.

L39 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 3

94043029. PubMed ID: 8226778. Kinetic analysis of ouabain binding to native and mutated forms of Na,K-ATPase and identification of a new region involved in cardiac glycoside interactions. Schultheis P J; Wallick E T; Lingrel J B. (Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati College of Medicine, Ohio 45267-0524.) Journal of biological chemistry, (1993 Oct 25) 268 (30) 22686-94. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cardiac glycosides inhibit the Na,K-ATPase by binding to the catalytic alpha subunit of the **enzyme**. Site-directed mutagenesis of the H1-H2 domain has demonstrated the importance of this region in determining cardiac glycoside affinity. In this study, random mutagenesis was used to identify an amino acid, arginine 880, in the COOH-terminal portion of the alpha subunit which influences the sensitivity of the **enzyme** to ouabain. This residue is predicted to reside in the H7-H8 extracellular loop. Conversion of arginine 880 to a proline causes a 10-fold **increase** in the dissociation rate constant and a 2-fold **increase** in the **association rate** constant for [3H]ouabain binding. This results in an **enzyme** with a KD for ouabain 5-fold higher than the wild-type sheep alpha 1 isoform. These data are compatible with arginine 880 comprising a portion of the ouabain binding site. Furthermore, if arginine 880 is at the physical binding site, then this finding lends support to models that place this amino acid extracellularly since cardiac glycosides interact with the extracellular surface of the Na,K-ATPase. The ouabain binding characteristics of substitution R880P were compared with those of several different Na,K-ATPases, each of which contains a single amino acid substitution in the H1-H2 region of the alpha subunit. The substituted **enzymes**, C104A, Y108A, E116Q, P118K, and Y124F, vary considerably in their rates of dissociation (1-**4-fold increase** in the dissociation rate constant). In addition, the rate of association of [3H]ouabain binding to substitution P118K is 2-fold slower than that of the wild-type **enzyme**. These results suggest that the H1-H2 domain may participate directly in ouabain binding as well as be involved in conformational changes, both of which could affect the sensitivity of the **enzyme** to ouabain.

L39 ANSWER 4 OF 4 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 4

92296013 EMBASE Document No.: 1992296013. Heparin-induced conformational change and activation of mucus proteinase inhibitor. Faller B.; Mely Y.; Gerard D.; Bieth J.G.. Faculte de Pharmacie, INSERM U 237, 74 route du Rhin,F-67400 Illkirch, France. Biochemistry Vol. 31, No. 35, pp. 8285-8290 1992.
ISSN: 0006-2960. CODEN: BICHAW
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 921025

AB Low molecular mass heparin (5.1 kDa) forms a tight complex with mucus proteinase inhibitor, the physiologic neutrophil elastase inhibitor of the upper respiratory tract. This binding strongly enhances the intrinsic fluorescence of the inhibitor and the rate of neutrophil elastase inhibitor association. One mole of this heparin fragment binds 1 mol of inhibitor with a K(d) of 50 nM. From the variation of K(d) with ionic strength, it is inferred that (i) 85% of the heparin-inhibitor binding energy is due to electrostatic interactions, (ii) about seven ionic interactions are involved in heparin-inhibitor binding, and (iii) about one-third of the ionized charges of heparin and inhibitor are involved in the complex. Heparin **4-fold increases** the very low quantum yield of Trp30, the single tryptophan residue of the inhibitor, blue-shifts its maximum emission wavelength by 6 nm, decreases the acrylamide quenching rate constant by a factor of 4, and **increases** the mean intensity weighted lifetime by a factor of 2.5. These important spectroscopic changes evidence a heparin-induced conformational change of the inhibitor which buries Trp30 in a very

hydrophobic environment. Heparin accelerates the inhibition of elastase in a concentration-dependent manner. When both **enzyme** and inhibitor are saturated by the polymer, the second-order **association rate** constant is 7.7 x 107 M-1 s-1, a value that is 27-fold higher than that measured with the free partners. This finding may have important physiologic and therapeutic bearing.

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L40 250 (HUSE W?/AU)

=> s l40 and association rate
L41 10 L40 AND ASSOCIATION RATE

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L42 6 DUP REMOVE L41 (4 DUPLICATES REMOVED)

=> d l42 1-6 cbib abs

L42 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN
2005:1089103 Ultra-potent antibodies against respiratory syncytial virus:. Wu, H.; Pfarr, D. S.; Tang, Y.; An, L-L.; Pate, N. K.; Watkins, J. D.; **Huse, W. D.**; Kiener, P. A.; Young, J. F. (USA). Assay and Drug Development Technologies, 3(4), 450-452 (English) 2005. CODEN: ADDTAR. ISSN: 1540-658X. Publisher: Mary Ann Liebert, Inc..

AB We describe there the selection of ultra-potent anti-respiratory syncytial virus (RSV) antibodies for preventing RSV infection. A large number of antibody variants derived from Synagis (palivizumab), an anti-RSV monoclonal antibody that targets RSV F protein, were generated by a directed evolution approach that allowed convenient manipulation of the binding kinetics. Palivizumab variants with about 100-fold slower dissociation rates or with fivefold faster **association rates** were identified and tested for their ability to neutralize virus in a microneutralization assay. Our data reveal a major differential effect of the association and dissociation rates on the RSV neutralization, particularly for intact antibodies wherein the **association rate** plays the predominant role. Furthermore, we found that antibody binding valence also plays a critical role in mediating the viral neutralization through a mechanism that is likely unrelated to antibody size or binding avidity. We applied an iterative mutagenesis approach, and thereafter were able to identify palivizumab Fab variants with up to 1,500-fold improvement and palivizumab IgG variants with up to 44-fold improvement in the ability to neutralize RSV. These anti-RSV immunoprophylaxis. In addition, our findings provide insights into engineering potent antibody therapeutics for other disease targets.

L42 ANSWER 2 OF 6 MEDLINE on STN DUPLICATE 1
2005293960. PubMed ID: 15907931. Ultra-potent antibodies against respiratory syncytial virus: effects of binding kinetics and binding valence on viral neutralization. Wu Herren; Pfarr David S; Tang Ying; An Ling-Ling; Patel Nita K; Watkins Jeffry D; **Huse William D**; Kiener Peter A; Young James F. (MedImmune, Inc., One MedImmune Way, Gaithersburg, MD 20878, USA.. wuh@medimmune.com) . Journal of molecular biology, (2005 Jul 1) 350 (1) 126-44. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB We describe here the selection of ultra-potent anti-respiratory syncytial virus (RSV) antibodies for preventing RSV infection. A large number of antibody variants derived from Synagis (palivizumab), an anti-RSV monoclonal antibody that targets RSV F protein, were generated by a directed evolution approach that allowed convenient manipulation of the binding kinetics. Palivizumab variants with about 100-fold slower dissociation rates or with fivefold faster **association rates** were identified and tested for their ability to neutralize

virus in a microneutralization assay. Our data reveal a major differential effect of the association and dissociation rates on the RSV neutralization, particularly for intact antibodies wherein the **association rate** plays the predominant role. Furthermore, we found that antibody binding valence also plays a critical role in mediating the viral neutralization through a mechanism that is likely unrelated to antibody size or binding avidity. We applied an iterative mutagenesis approach, and thereafter were able to identify palivizumab Fab variants with up to 1500-fold improvement and palivizumab IgG variants with up to 44-fold improvement in the ability to neutralize RSV. These anti-RSV antibodies likely will offer great clinical potential for RSV immunoprophylaxis. In addition, our findings provide insights into engineering potent antibody therapeutics for other disease targets.

L42 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

2004:550529 Document No. 141:87793 Recombinant humanized anti-TNFα monoclonal antibody Fab fragments with higher affinity to TNFα for treatment of TNFα-mediated diseases. Watkins, Jeffry D.; Vasserot, Alain P.; Marquis, David; **Huse, William D.** (USA). U.S. Pat. Appl. Publ. US 2004131613 A1 20040708, 60 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-338627 20030108.

AB The present invention relates to TNF-α binding mols. and nucleic acid sequences encoding TNF-α binding mols. In particular, the present invention relates to TNF-α binding mols. with a high binding affinity, a high **association rate**, a low dissociation rate with regard to human TNF-α and that are capable of neutralizing TNF-α at low concns. Preferably, the TNF-α binding mols. of the present invention comprise light and/or heavy chain variable regions with fully human frameworks (e.g. human germline frameworks). Specifically described are construction and screening of anti-TNF-α Fab fragments with synthetic CDRs and human frameworks based on the hu1 antibody. The mutations of these recombinant anti-TNF-α Fab fragments include 2C6K:S31L/S54Y in light chain, and T28K/E61R in heavy chain; 2C6P:S31L/S54Y in light chain, and T28P/E61R in heavy chain; 2E7K:S31L/M55R in light chain, and T28K/I53M/E61R in heavy chain; 2E7P:S31/ M55R in light chain, and T28P/I53M/E61R; A9K:S31M/M55K in light chain, and T28K/I53M/E61R in heavy chain; A9P:S31M/M55K in light chain, and T28P/I53M/E61R in heavy chain; A10K:S31Y/M55R in light chain, and T28K/I53M in heavy chain; and A10P:S31Y/M55R in light chain, and T28P/I53M in heavy chain.

L42 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

2004:550528 Document No. 141:87792 Recombinant humanized anti-TNFα monoclonal antibody Fab fragments for the treatment of TNFα-mediated diseases. Watkins, Jeffry D.; Vasserot, Alain P.; Marquis, David; **Huse, William D.** (USA). U.S. Pat. Appl. Publ. US 2004131612 A1 20040708, 60 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-338552 20030108.

AB The present invention relates to TNF-α binding mols. and nucleic acid sequences encoding TNF-α binding mols. In particular, the present invention relates to TNF-α binding mols. with a high binding affinity, a high **association rate**, a low dissociation rate with regard to human TNF-α and that are capable of neutralizing TNF-α at low concns. Preferably, the TNF-α binding mols. of the present invention comprise light and/or heavy chain variable regions with fully human frameworks (e.g. human germline frameworks). Specifically described are construction and screening of anti-TNF-α Fab fragments with synthetic CDRs and human frameworks based on the hu1 antibody. The mutations of these recombinant anti-TNF-α Fab fragments include 2C6K:S31L/S54Y in light chain, and T28K/E61R in heavy chain; 2C6P:S31L/S54Y in light chain, and T28P/E61R in heavy chain; 2E7K:S31L/M55R in light chain, and T28K/I53M/E61R in heavy chain; 2E7P:S31/ M55R in light chain, and T28P/I53M/E61R; A9K:S31M/M55K in light chain, and T28K/I53M/E61R in heavy chain; A9P:S31M/M55K in light chain, and T28P/I53M/E61R in heavy chain; A10K:S31Y/M55R in light chain, and

T28K/I53M in heavy chain; and A10P:S31Y/M55R in light chain, and T28P/I53M in heavy chain.

L42 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN
2002:353476 Document No. 136:350534 Methods for producing and improving therapeutic potency of binding polypeptides. **Huse, William D.** (Applied Molecular Evolution, Inc., USA). PCT Int. Appl. WO 2002036615 A2 20020510, 82 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US46248 20011030. PRIORITY: US 2000-2000/702140 20001030.
AB The invention provides a binding polypeptide, or functional fragment thereof, comprising a kon of at least about 9 x 107 M-1 s-1 for associating with a ligand and having therapeutic potency. The invention also provides a method of determining the therapeutic potency of a binding polypeptide. The methods consist of (a) contacting a binding polypeptide with a ligand; (b) measuring **association rate** for binding between the binding polypeptide and the ligand, and (c) comparing the **association rate** for the binding polypeptide to an **association rate** for a therapeutic control, the relative **association rate** for the binding polypeptide compared to the **association rate** for the therapeutic control indicating that the binding polypeptide will exhibit a difference in therapeutic potency correlative with the difference between the **association rates.**

L42 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN
2001:661491 Document No. 135:240921 High potency recombinant antibodies and method for producing them. Young, James F.; Koenig, Scott; Johnson, Leslie S.; **Huse, William D.**; Wu, Herren; Watkins, Jeffry D. (Medimmune, Inc., USA). PCT Int. Appl. WO 2001064751 A2 20010907, 98 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US6815 20010301. PRIORITY: US 2000-PV186252 20000301.
AB High potency antibodies, including immunol. active fragments thereof, having high kinetic **association rate** consts. and optional high affinities are disclosed, along with methods for producing such antibodies. The high potency antibodies disclosed herein are of either the neutralizing or non-neutralizing type and have specificity for antigens displayed by microorganisms, especially viruses, as well as antigenic sites present on cancer cells and on various types of toxins, and the products of toxins. Processes for production high potency neutralizing antibodies and increasing the potency of already existing neutralizing antibodies are also described. Methods of using said antibodies in the prevention and/or treatment of diseases, especially diseases induced or caused by viruses, are disclosed.

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---Logging off of STN---

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1: Acta Endocrinol (Copenh). 1988 Feb;117(2):159-65.

Study of the role of histidyl, tyrosyl, alpha- or epsilon-amino residues in the specific binding of 3,5,3'-triiodothyronine to rat liver nuclear receptors.

Brtko J, Knopp J.

Institute of Experimental Endocrinology, Centre of Physiological Sciences, Bratislava, Czechoslovakia.

The role of histidyl, tyrosyl, alpha-or epsilon-amino residues of rat liver nuclear receptors for the specific binding of T3 was studied by chemically modifying the receptor molecule. The kinetics of the formation of N-carbethoxyhistidyl derivative from histidyl groups of nuclear receptors by diethylpyrocarbonate was examined. The modified nuclear receptor fraction was separated from diethylpyrocarbonate by gel filtration and the T3 binding parameters (Ka and MBC) at pH 8.0 were tested by Scatchard plot analysis. At 0.1 mmol/l diethypyrocarbonate, the value of Ka was significantly (P less than 0.01) decreased without any change in maximal binding capacity (MBC). The modification of alpha- or epsilon-amino groups of nuclear receptors by excess of trinitrobenzenesulfonic acid, 6.3 mmol/l at pH 8.5, resulted in a 4-fold increase in MBC of T3 specific binding without any change in Ka. In addition, acetylation of tyrosyl residues of nuclear receptors at pH 7.5 with an excess of 24 mmol/l N-acetylimidazole was performed. No changes in nuclear receptor Ka or MBC were observed after N-acetylimidazole treatment. Histidine and/or amino groups of the receptor molecule seem to hold a key position in the generation of the biologically active T3-nuclear receptor complex in the rat liver.

PMID: 3132789 [PubMed - indexed for MEDLINE]

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1: Neurochem Res. 1997 Apr;22(4):437-43. Related Articles, Links

Substitution of D-Trp32 in NPY destabilizes the binding transition state to the Y1 receptor site in SK-N-MC cell membranes.

Zand R, Marcelo CL, MacKenzie R, Georgic L, Maclean D, Dunham WR.

Department of Biological Chemistry, University of Michigan, Ann Arbor 48109, USA. rzand@umich.edu

The retention rate of the spin label 3-isothiocyanto methyl-2,2,5,5-tetramethyl-1-pyrrolidinyl oxyl spin label (proxyl) attached to the porcine N-acetyl-NPY peptide and the porcine N-acetyl-D-Trp32-NPY peptide at Lys4 was investigated using SK-N-MC neuroblastoma cell membranes containing the Y1 receptor. The release rate of the spin labeled peptides was monitored by electron spin resonance and the KD was determined by a direct radiolabeled NPY displacement binding assay. The analyses show that for the porcine [Ac-Tyr1N epsilon 4-proxyl]-NPY, the KD was 8 x 10(-10) M and koff was 2.7 x 10(-4) sec-1 yielding a value for kon of 3.3 x 10(5) sec-1 M-1. The [Ac-Tyr1, N epsilon 4-proxyl,-D-Trp32]-NPY antagonist ligand had a value of KD equal to 1.35 x 10(-7) M and koff was 1.7 x 10(-4) sec-1 leading to a value for kon of 1.2 x 10(3) sec-1 M-1. The difference in the kon rates of two orders of magnitude is interpreted as demonstrating the N-acetyl-N epsilon 4 proxyl-D-Trp32-NPY ligand binding transition state to be of higher energy then for the unmodified NPY amino acid sequence.

PMID: 9130254 [PubMed - indexed for MEDLINE]

1: Mol Pharmacol. 1990 Aug;38(2):214-21.

p-[125I]iodoclonidine is a partial agonist at the alpha 2-adrenergic receptor.

Gerhardt MA, Wade SM, Neubig RR.

Department of Pharmacology, University of Michigan Medical School, Ann Arbor 48109-0626.

The binding properties of p-[125I]iodoclonidine [(125I]PIC) to human platelet membranes and the functional characteristics of PIC are reported. [125I]PIC bound rapidly and reversibly to platelet membranes, with a first-order association rate constant (kon) at room temperature of 8.0 +/- 2.7 x 10 (6) M-1 sec-1 and a dissociation rate constant (koff) of 2.0 +/- 0.8 x 10(-3) sec-1. Scatchard plots of specific [125I]PIC binding (0.1-5 nM) were linear, with a Kd of 1.2 +/- 0.1 nM. [125I]PIC bound to the same number of high affinity sites as the alpha 2-adrenergic receptor (alpha 2-AR) full agonist [3H] bromoxidine (UK14,304), which represented approximately 40% of the sites bound by the antagonist [3H]yohimbine. Guanosine 5'-(beta, gamma-imido)triphosphate greatly reduced the amount of [125I]PIC bound (greater than 80%), without changing the Kd of the residual binding. In competition experiments, the alpha 2-AR-selective ligands yohimbine, bromoxidine, oxymetazoline, clonidine, p-aminoclonidine, (-)-epinephrine, and idazoxan all had Ki values in the low nanomolar range, whereas prazosin, propranolol, and serotonin yielded Ki values in the micromolar range. Epinephrine competition for [125I]PIC binding was stereoselective. Competition for [3H]bromoxidine binding by PIC gave a Ki of 1.0 nM (nH = 1.0), whereas competition for [3H]yohimbine could be resolved into high and low affinity components, with Ki values of 3.7 and 84 nM, respectively. PIC had minimal agonist activity in inhibiting adenylate cyclase in platelet membranes, but it potentiated platelet aggregation induced by ADP with an EC50 of 1.5 microM. PIC also inhibited epinephrine-induced aggregation, with an IC50 of 5.1 microM. Thus, PIC behaves as a partial agonist in a human platelet aggregation assay. [125I]PIC binds to the alpha 2B-AR in NG-10815 cell membranes with a Kd of 0.5 +/- 0.1 nM. [125I]PIC should

prove useful in binding assays involving tissues with a low receptor density or in small tissue samples and in studies of cloned and expressed alpha 2-AR.

PMID: 1974694 [PubMed - indexed for MEDLINE]

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1: Hybridoma. 2000 Apr;19(2):135-42. Related Articles, Links

A monoclonal antibody directed against an autoimmune epitope on the human beta1-adrenergic receptor recognized in idiopathic dilated cardiomyopathy.

Mobini R, Fu M, Wallukat G, Magnusson Y, Hjalmarson A, Hoebeke J.

Wallenberg Laboratory for Cardiovascular Research, Sahlgrenska University Hospital, Gothenburg, Sweden. reza@mobini@wlab.wall.gu.se

A monoclonal antibody (MAb M16) was obtained by immunizing Balb/C mice with free peptide H26R, corresponding to the second extracellular loop of the human beta1-adrenergic receptor (beta1AR), against which functional autoantibodies have been detected in patients with idiopathic dilated cardiomyopathy. The MAb was found to be of IgG2b type and directed against a conformational epitope, encompassing the sequence recognized by the human autoantibodies. BIAcore measurements yielded an equilibrium constant of 6.5 X 10(7) M1 with an association rate constant (kon) of 6.5 X 10(4) M(-1) sec(-1) and a dissociation rate constant (koff) of 1.0 X 10(-3) sec(-1). It immunoprecipitated only poorly the solubilized beta1AR of Sf9 cell membranes. Functionally, the MAb was capable of not only reducing the number of the maximal binding sites to the beta1-adrenergic receptor of transfected Sf9 cell membranes, but also of displaying a positive chronotropic effect on cultured neonatal rat cardiomyocytes. These properties, which the MAb shares with the human autoantibodies, makes it an interesting tool for passive transfer studies in mice.

PMID: 10868793 [PubMed - indexed for MEDLINE]